

For Reference

NOT TO BE TAKEN FROM THIS ROOM

For Reference

NOT TO BE TAKEN FROM THIS ROOM

Ex LIBRIS UNIVERSITATIS ALBERTAENSIS



UNIVERSITY OF ALBERTA
LIBRARY

Regulations Regarding Theses and Dissertations

Typescript copies of theses and dissertations for Master's and Doctor's degrees deposited in the University of Alberta Library, as the official Copy of the Faculty of Graduate Studies, may be consulted in the Reference Reading Room only.

A second copy is on deposit in the Department under whose supervision the work was done. Some Departments are willing to loan their copy to libraries, through the inter-library loan service of the University of Alberta Library.

These theses and dissertations are to be used only with due regard to the rights of the author. Written permission of the author and of the Department must be obtained through the University of Alberta Library when extended passages are copied. When permission has been granted, acknowledgement must appear in the published work.

This thesis or dissertation has been used in accordance with the above regulations by the persons listed below. The borrowing library is obligated to secure the signature of each user.

Please sign below:



Digitized by the Internet Archive
in 2023 with funding from
University of Alberta Library

<https://archive.org/details/CHarley1969>

1969
1969
94

THE UNIVERSITY OF ALBERTA

THE DIETARY AND PHARMACOLOGIC MANIPULATION
OF SERUM LIPOPROTEINS IN SUBJECTS WITH AND
WITHOUT CORONARY ARTERY DISEASE

by



Charles H. Harley

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

DEPARTMENT OF MEDICINE

EDMONTON, ALBERTA

FALL, 1969

UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read,
and recommend to the Faculty of Graduate Studies for
acceptance, a thesis entitled " The Dietary and
Pharmacologic Manipulation of Serum Lipoproteins in
Subjects with and without Coronary Artery Disease "
submitted by Charles Herbert Harley in partial
fulfilment of the requirements for the degree of
Master of Science.

ABSTRACT

There were two major aspects to this investigation. The first was to study a group of young males with documented premature coronary artery disease for abnormalities of serum lipids, lipoproteins and carbohydrate-inducibility of endogenous hypertriglyceridemia. To rapidly reveal this latter phenomenon, these individuals were challenged with a seven day high carbohydrate diet. Twenty-one patients under 50 years of age were selected and following assessment of serum lipids, lipoproteins (by paper and agarose-gel electrophoresis), and glucose tolerance, these individuals were placed on an 85% high carbohydrate diet for seven days. Serum lipids and lipoproteins were repeated during and at the completion of the diet. Nine healthy males under 30 years of age were studied in a similar manner. Young controls were chosen to reduce the possibility of the presence of latent lipid or carbohydrate abnormalities or asymptomatic vascular disease.

The patient group showed significantly higher serum triglycerides than the control group before, and at the completion of, the dietary study ($p < 0.01$). Also of interest was the observation that almost 50% of the patients had a lipoprotein abnormality of some type even before the dietary challenge. A large proportion of the patients

(66%) demonstrated the phenomenon of carbohydrate-inducibility. It was concluded, therefore, that a large number of young patients with coronary artery disease convert dietary carbohydrate into triglycerides to an abnormal degree, and that the seven day period of high carbohydrate feeding was sufficient to demonstrate this abnormality.

The second purpose of this investigation was to study the effect of clofibrate on serum lipids and lipoproteins of normal males and to evaluate its effect, if any, on serum insulin, glucose tolerance and serum free fatty acids. The investigation was conducted in two parts. First, nine healthy males between 29 - 49 years of age were selected. Following baseline lipid studies, assessment of plasma glucose and serum insulin responses to oral glucose administration were performed. Clofibrate was then administered in therapeutic doses for 30 days. At the conclusion of the 30 day period, the tests were repeated. Secondly, eight healthy males were studied in a manner similar to that outlined above, except that glucose was administered intravenously at a constant rate. In addition, serum free fatty acids were also assessed. These subjects were then placed on clofibrate for a period of 14 days and the tests repeated.

From these studies, it was concluded that clofibrate lowers serum triglycerides in normal males to a significant degree ($p < 0.05$). No effect was noted on serum insulin, glucose tolerance or serum free fatty acids.

Lastly, it was obvious from these investigations that agarose-gel electrophoresis is able to reveal subtle changes in pre-beta lipoproteins that would go unnoticed by paper electrophoresis. Thus, it is the method of choice in such studies.

ACKNOWLEDGEMENTS

I wish to express my thanks to the many individuals who, in various ways, have helped and advised me in carrying out this project. To Dr. R. S. Fraser and Dr. D. J. Campbell I cannot adequately express my deep appreciation for the opportunity of spending a year under their guidance and for the constant help and encouragement that they gave me. I am also grateful to Dr. P. Crockford for many valuable suggestions and criticisms, and to the physicians in the Department of Medicine for their interest in selecting suitable subjects for the study.

It is a pleasure to acknowledge the assistance provided by many people in the Department of Clinical Laboratories, particularly Mrs. J. Dakin and Mr. R. Baynton. I am also indebted to Miss C. Lalonde who carried out the serum insulin assays.

I am also grateful to the Metabolic Dietician, Miss L. Cotsman for her assistance in the preparation and administration of the diet.

This work was carried out in the Department of Clinical Laboratories, University of Alberta Hospital, and appreciation is extended to Dr. R. E. Bell, Director, for use of the facilities.

The author is grateful to Miss S. Bennett for the

typing of the manuscript.

The financial support of the Alberta Heart Foundation and Ayerst Laboratories is also gratefully acknowledged.

Edmonton, Alberta
June, 1969

Charles H. Harley

TABLE OF CONTENTS

CHAPTER	PAGE
I	Introduction.....1
II	Background Literature.....6
	A. The Relationship of Elevated Serum Lipids to Atherosclerotic Heart Disease.....6
	B. Carbohydrate Abnormalities and Atheroscler- otic Heart Disease.....13
	C. Carbohydrate Induction Studies.....17
	D. The Plasma Lipoproteins.....21
	E. The Hyperlipoproteinemias.....27
	F. Methods Used in Lipoprotein Separation.....33
	G. Clofibrate.....37
III	Methods and Procedures.....44
	A. Methods.....44
	1) Paper Electrophoresis.....44
	2) Agarose-Gel Electrophoresis.....46
	3) Serum Cholesterol.....51
	4) Serum Triglycerides.....51
	5) Glucose Tolerance Tests.....52
	6) Immunoassay of Insulin.....53
	7) Serum Free Fatty Acids.....54
	B. Dietary Studies.....54
	1) Selection of Patients and Controls.....54
	2) Diet.....55

CHAPTER	PAGE
3) Lipid and Carbohydrate Studies.....	56
C. Clofibrate Studies.....	56
1) Selection of Subjects.....	56
2) Lipids, Carbohydrate and Insulin Studies.....	56
IV Results.....	59
A. The Effect of a Seven Day High Carbohydrate Diet on Serum Lipids in Normal Subjects and Patients with Coronary Artery Disease.....	59
1) Serum Triglycerides.....	64
2) Serum Cholesterol.....	67
3) Serum Lipoproteins.....	69
4) The Effect of a Seven Day High Carbohyd- rate Diet on Serum Lipoprotein Fractions as Separated by Agarose-Gel Electrophor- esis and Quantitated by Densitometric Scanning.....	80
B. The Effects of Clofibrate in Normal Subjects.....	89
1) Serum Triglycerides.....	89
2) Serum Cholesterol.....	92
3) Serum Lipoproteins.....	92
4) Changes in Lipoprotein Fractions as Revealed by Densitometric Scanning of Agarose-Gel Electropherograms.....	96
5) The Effect of Thirty Days of Clofibrate Administration on Serum Insulin Levels During Oral Glucose Challenge.....	99
6) The Effect of Thirty Days of Clofibrate Administration on Serum Insulin Levels During Oral Glucose Challenge.....	99

CHAPTER	PAGE
7) Plasma Glucose Studies After Fourteen Days of Clofibrate Administration During Glucose Infusion.....	103
8) Serum Insulin Levels Before and After Clofibrate Administration for Fourteen Days.....	104
9) Free Fatty Acid Levels Before and After Clofibrate Administration for Fourteen Days.....	104
10) Serum Triglycerides, Cholesterol and Lipoproteins After Fourteen Days of Clofibrate Administration.....	110
V. Discussion.....	119
A. Dietary Studies.....	119
B. Clofibrate Studies.....	125
VI. Summary.....	129
VII. Appendix.....	133
VIII. Bibliography.....	134

LIST OF TABLES

TABLE	PAGE
1	The Effects of a Seven Day High Carbohydrate Diet on the Serum Lipids of Control Subjects.....60
2	The Effect of a Seven Day High Carbohydrate Diet on Serum Lipids of Young Patients with Coronary Artery Disease.....61
3	Average Effect of a Seven Day High Carbohydrate Diet on Serum Triglycerides.....65
4	Average Effect of a Seven Day High Carbohydrate Diet on Serum Cholesterol.....65
5	The Effect of a Seven Day High Carbohydrate Diet on the Serum Lipoproteins of Control Subjects.....70
6	The Effect of a Seven Day High Carbohydrate Diet on Serum Lipoproteins of Young Patients with Coronary Artery Disease.....71
7	Effect of a Seven Day High Carbohydrate Diet on the Serum Lipoprotein Fractions of Controls as Separated by Agarose-Gel Electrophoresis and Quantitated by Densitometric Scanning.....81
8	Effect of a Seven Day High Carbohydrate Diet on the Serum Lipoprotein Fractions of Young Patients with Coronary Artery Disease as Separated by Agarose-Gel Electrophoresis and Quantitated by Densitometric Scanning.....82
9	Changes in the Ratio of Pre-beta to other Lipoprotein Fractions Due to High Carbohydrate Diet...86
10	Changes in the Ratio of Beta to Other Lipoprotein Fractions Due to High Carbohydrate Diet.....86
11	Changes in the Ratio of Alpha to Other Lipoprotein Fractions Due to High Carbohydrate Diet.....86
12	The Effect of Thirty Days of Clofibrate on the Serum Lipids of Normal Subjects.....90
13	Average Effect of Thirty Days of Clofibrate on the Serum Triglycerides of Normal Subjects.....91
14	Average Effect of Thirty Days of Clofibrate on the Serum Cholesterol of Normal Subjects.....91

TABLE	PAGE
15	The Effects of Thirty Days of Clofibrate on the Serum Lipoproteins of Normal Subjects.....93
16	Effect of Thirty Days of Clofibrate on Serum Lipoprotein Fractions as Separated by Agarose-Gel Electrophoresis and Quantitated by Densitometric Scanning.....97
17	Changes in the Ratio of Pre-Beta to Other Lipoprotein Fractions After Thirty Days of Clofibrate.98
18	Changes in the Ratio of Beta to Other Lipoprotein Fractions After Thirty Days of Clofibrate.....98
19	Changes in the Ratio of Alpha to Other Lipoprotein Fractions After Thirty Days of Clofibrate.....98
20	Average Effect of Thirty Days of Clofibrate Administration on Plasma Glucose and Serum Insulin Levels During Oral Glucose Challenge.....100
21	Effect of Fourteen Days of Clofibrate Administration on Plasma Glucose and Serum Insulin Levels During Intravenous Infusion of Glucose.....105
22	Effect of Fourteen Days of Clofibrate Administration on Free Fatty Acid Levels During Intravenous Infusion of Glucose.....108
23	The Effect of Fourteen Days of Clofibrate on the Serum Lipids of Normal Subjects.....111
24	Average Effect of Fourteen Days of Clofibrate on the Serum Triglycerides of Normal Subjects.....112
25	Average Effect of Fourteen Days of Clofibrate on the Serum Cholesterol of Normal Subjects.....112
26	The Effects of Fourteen Days of Clofibrate on the Serum Lipoproteins of Normal Subjects.....114
27	Effect of Fourteen Days of Clofibrate on Serum Lipoprotein Fractions as Separated by Agarose-Gel Electrophoresis and Quantitated by Densitometric Scanning.....116

TABLE	PAGE
28	Changes in the Ratio of Pre-beta to Other Lipoprotein Fractions after Fourteen Days of Clofibrate.....117
29	Changes in the Ratio of Beta to Other Lipoprotein Fractions after Fourteen Days of Clofibrate.....117
30	Changes in the Ratio of Alpha to Other Lipoprotein Fractions after Fourteen Days of Clofibrate.....117

LIST OF FIGURES

FIGURE	PAGE
1	The Hyperlipoproteinemias (Type I - V) by Paper Electrophoresis.....29
2	Effect of a High Carbohydrate on the Serum Triglycerides (T.G.) of Controls and Patients with Coronary Artery Disease.....66
3	Effect of a High Carbohydrate Diet on the Serum Cholesterol of Controls and Patients with Coronary Artery Disease.....68
4	Normal and Type II Hyperlipoproteinemia by Paper Electrophoresis.....76
5	Type IV Hyperlipoproteinemia Before and After Seven Days of a High Carbohydrate Diet.....77
6	Normal and Type II Hyperlipoproteinemia by Agarose-Gel Electrophoresis78
7	Type IV Hyperlipoproteinemia by Agarose-Gel Electrophoresis Before and After Seven Days of a High Carbohydrate Diet.....79
8	Glucose Tolerance Index of Patients with Coronary Artery Disease.....88
9	Effect of 30 Days of Clofibrate on Serum Lipoproteins Separated by Agarose-Gel Electrophoresis.....95
10	Effect of Clofibrate on Oral Glucose Tolerance....101
11	Effect of Clofibrate on Serum Insulin Levels After Oral Glucose Administration.....102
12	Effect of Clofibrate on Intravenous Glucose Administration.....106
13	Effect of Clofibrate on Serum Insulin Levels During Intravenous Glucose Infusion.....107
14	Effect of Clofibrate on Serum FFA's During Intravenous Glucose Infusion.....109

INTRODUCTION

Coronary artery disease and atherosclerosis in young adults are accounting for an increasing morbidity and mortality in economically well-developed countries. A number of studies have been devoted to identifying factors which may aid in distinguishing susceptible members of the population, and have revealed the following:

1. There appear to be a number of "risk factors".
2. Combinations of these "risk factors" rather than any single factor provide the best prediction of susceptibility.
3. These "risk factors" are present long before the disease becomes clinically manifest.

Although the etiology of atherosclerosis and coronary artery disease remains unclear, most of the commonly accepted theories, although multifactorial, implicate serum lipids to a greater or lesser degree. Indeed, Marchand¹, who first used the term atherosclerosis, suggested that the lipids contained in the plaques so characteristic of the disease, were derived from the blood by a process of filtration.

Considerable evidence has been accumulated relating nutrition to atherosclerosis; presumably through its influence on the level of circulating lipids. The relationship of dietary factors and serum lipids to the subsequent develop-

ment of the disease has been investigated through animal experiments, clinical observations and international epidemiological studies. Most of this work indicates that the populations in economically well-developed countries consume diets which promote over-nutrition, and which are high in total calories, dairy fats, total fat, saturated fat, cholesterol and refined carbohydrates. People in these countries tend to have higher serum lipids and demonstrate a higher incidence of atherosclerosis both clinically and pathologically. In contrast, people from less economically well-developed areas consume diets which are low in total calories with few foods of animal origin and less total fat, saturated fat, cholesterol and refined sugars. Serum lipid levels tend to be lower and remain so throughout life. Coronary atherosclerosis, both clinical and pathological, is a rare occurrence.

Carbohydrates and fats, which together contribute 85% of total caloric intake, have attracted the greatest attention as major factors influencing blood lipids. Opinions regarding the etiology of atherosclerosis in recent years, have swung away from a mechanistic explanation which suggested that ingested cholesterol and fats are the principle factors in the process to one which accepts a basic systemic metabolic abnormality. Increasing evidence suggests that

an important and perhaps basic defect lies in the area of carbohydrate metabolism. Many observations have shown that patients with the carbohydrate-inducible form of hyperglyceridemia have defective release or utilization of insulin. These findings more closely unite the long known association between diabetes and coronary artery disease. Elevated serum lipids and abnormalities of carbohydrate metabolism are considered to be important metabolic indicators of susceptibility to atherosclerosis.

Because of the multiplicity of metabolic factors involved in the process, it is evident that measurement of serum cholesterol is not sufficient in the investigation of patients with atherosclerosis. Today, this must include determinations of serum triglycerides, serum lipoproteins, and some assessment of carbohydrate metabolism.

The qualitative evaluation of serum lipoproteins by electrophoresis in patients with coronary artery disease is carried out through the use of several techniques including paper, starch block, cellulose acetate, and more recently agarose gel. The latter technique, in particular, shows increasing promise particularly in the evaluation of changes in pre-beta lipoproteins (very low density lipoproteins, VLDL).

The search for a pharmacologic agent which is safe, specific and effective for an indefinite period in lowering serum lipids has been intensified. It is highly unlikely, however, that any type of drug therapy can ever take the place of dietary modifications in the general population. Clofibrate (p-chlorophenoxyisobutyrate) has been shown to be effective in lowering serum triglycerides and cholesterol in clinical trials. Its effects are more consistent in relation to the former, which is the fraction elevated in the carbohydrate-inducible forms of hyperlipidemia.

There is growing evidence of a causal relationship between carbohydrate inducibility of triglycerides and premature atherosclerosis. Thus, it was decided to investigate a group of subjects with premature vascular disease for abnormalities in serum lipoproteins (particularly the VLDL), carbohydrate intolerance and carbohydrate inducibility by dietary challenge during a period of carbohydrate feeding. Formerly, this required long-term metabolic studies (weeks or months) to establish steady-state conditions. As this is not possible in an active treatment hospital, such a challenge was modified to consist of a seven day period. It was hoped that this would be sufficient to

reveal an abnormal change in lipids.

The shortcomings of paper electrophoresis in qualitative evaluation of VLDL prompted, in addition, the use of agarose gel electrophoresis. Others have shown that this method gives excellent qualitative results in the evaluation of subtle changes of the VLDL fraction. Furthermore it permits semi-quantitation of lipoprotein fractions by densitometric scanning.

As noted above, clofibrate has been reported to be most effective in hypertriglyceridemic states. However, the mechanism of this effect is still unclear. Because of the relationship of VLDL to carbohydrate ingestion and insulin, it was decided to study the effect of clofibrate on serum insulin levels, as well as serum lipids and lipoproteins. Unlike previous studies, normal individuals with no overt evidence or tendency toward coronary artery disease were selected.

BACKGROUND LITERATURE

A. THE RELATIONSHIP OF ELEVATED SERUM LIPIDS TO ATHEROSCLEROTIC HEART DISEASE.

The overwhelming accumulation of evidence in the study of atherosclerosis would indicate that the disease is of multifactorial etiology and no single cause can be designated. However, most of these studies have indicated that serum lipids play a key role in the genesis and perpetuation of the condition.

Cholesterol

The evidence relating atherosclerosis to abnormalities of serum lipids has been derived from three areas of medical research. These include experimental, clinical and epidemiological studies.

- i) Experimental - In the early twentieth century,
2
Anitschkow , demonstrated that rabbits fed animal tissues developed elevated serum cholesterol and generalized atherosclerosis. Other dietary studies in laboratory animals have confirmed this fact although the analogies relating to the human being are not as clear-cut. The importance of these studies, however, lies in the observation that dietary factors alone can accelerate the development of the disease. Studies in animals, more pertinent

to the problem in humans, have revealed that subtle changes in the amounts and duration of diets containing cholesterol and fat can produce more moderate hypercholesterolemia and atherosclerosis. This feature is highly analogous to the situation in the human populations in economically well-developed societies.

- ii) Clinical studies - A number of disease states are associated with the premature development of atherosclerosis. These include diabetes mellitus, nephrotic syndrome, hypothyroidism, and familial hypercholesterolemia. All of these abnormalities are associated with an underlying metabolic disorder with disruption of neural-hormonal-enzyme systems. These findings substantiate the basis of the primary disease as a systemic metabolic disorder, with exogenous lipid as a key, but not an exclusive, factor.
- iii) Epidemiologic studies - The suggestion that elevation of serum cholesterol was related to the development of coronary artery disease was inferred from two broad areas of investigation.
 - a) Studies of patients with arteriosclerotic

heart disease have revealed elevated levels of serum cholesterol as compared to "normal" controls.

- b) Patients with genetically determined elevations of serum cholesterol develop atherosclerosis at a very early age and to a more severe degree.

It has been abundantly demonstrated and unequivocally proved in most epidemiological surveys that the risk of experiencing a clinical episode of coronary artery disease is a function, in part, of serum cholesterol levels.

³
Kannel et al. , studied a mixed population of males between the ages of 30 and 59 years over a ten year period, for the development of coronary artery disease. These studies revealed that hypercholesterolemia preceeded the development of the disease and was associated with its development. He concluded that the elevation of serum cholesterol over 245 mg/100 ml was associated with a three-fold increase in the risk of development of the disease. In addition, the risk of sudden death was related to an antecedent elevation of serum cholesterol above normal.

⁴
Chapman et al. , studied 1503 patients over a ten year period for the development of coronary artery disease. Forty-four percent of the subjects studied had cholesterol values over 300 mg/100 ml (normal 270 mg/100 ml). He concluded that the highest incidence of myocardial infarction occurred among those with the highest cholesterol levels. He related the incidence of myocardial infarction per 1000 population to the level of serum cholesterol;

<u>Serum Cholesterol (mgm %)</u>	<u>Incidence of Myocardial Infarction per 1000 population</u>
less than 210 mgm %	21.6
210 - 269 mgm %	30.3
270 - 389 mgm %	82.1
greater than 390 mgm %	117.6

⁵
Keys et al. , studied 281 men for fifteen years and considered a number of parameters. He concluded that the most significant factor related to the development of the disease was the serum cholesterol ($p < 0.001$).

⁶
Paul et al. , studied 1989 men for a five year period. The most significant conclusion drawn was the stepwise association of the serum cholesterol to the increasing incidence of coronary artery disease.

⁷
Epstein , concluded that available epidemiological evidence demonstrated that the incidence of coronary artery disease increased as the serum cholesterol increased, but that the risk applied to groups and not necessarily to individuals. In commenting on international epidemiological studies, the same author concluded that differences in population sampling and methodology created some difficulties in comparing these studies, but that a clear-cut association between coronary artery disease and serum cholesterol has been demonstrated, and that the morbidity and mortality from the disease tends to be related to the average serum cholesterol levels in the affected population.

Triglycerides

⁸
Beginning with the work of Gofman et al. , increasing emphasis has been placed on the role of serum triglycerides, and more particularly, the triglyceride-rich VLDL

($S_f^* \cdot 20 - 400$) as better indicators of risk to the subsequent development of coronary artery disease. The significance of this approach has been seriously questioned by Brown et al.⁹, and Doyle¹⁰, who found that triglyceride levels had no greater prognostic significance in the epidemiology of atherosclerotic vascular disease. Much of the dissent, concerning the significance of triglycerides as metabolic indicators has been due to the necessity of obtaining fasting samples from large groups. In addition, until recently, triglyceride measurements have been cumbersome and time-consuming.

Albrink and Man¹¹, in a study of patients with myocardial infarction, found that serum triglycerides above 160 mg/100 ml were present in 85% of 100 patients between 20 and 78 years of age with coronary artery disease as compared to only 5% of young non-affected males between 20 and 30 years of age and in 30% of males over 50 years of age. Serum cholesterol was abnormal in only 18% of patients with coronary artery disease.

* S_f Units (Svedburgs of Floatation)
One Svedburg unit equals 10^{-13} cm/sec/dyne/q.

¹²
Albrink , again reaffirmed her belief that triglycerides were a better measure of susceptibility to atherosclerosis and stated that elevations of triglycerides, alone or in combination with elevations of cholesterol, was the most common abnormality of lipids in coronary artery disease.

¹³
Carlson , studied 49 patients with myocardial infarction between 33 and 65 years of age. He noted that the mean serum cholesterol was significantly elevated in the observed population, when compared to control subjects, and a significant increase in triglycerides was present in the two lowest age groups. He concluded from the study that in men under 50 years of age with myocardial infarction, frequent elevations of triglycerides were noted, whereas, after that age the main finding was an elevated serum cholesterol.

¹⁴
Havel and Carlson , concluded that the level of serum triglycerides correlated more closely than cholesterol as an indicator of atherosclerosis, particularly in the younger age groups although discrepancies existed over the age of 50. They indicated that neither measurement alone provided adequate information for the evaluation of lipid patterns, since both fractions may be elevated either alone or in combination.

B. CARBOHYDRATE ABNORMALITIES AND ARTERIOSCLEROTIC HEART DISEASE.

The role of lipids in the production of atherosclerosis is now well established. Recent studies, however, have laid emphasis on sub-clinical abnormalities in carbohydrate metabolism¹⁵, as having physiological significance in the genesis of coronary artery disease.

Carbohydrate and lipid metabolism are closely related and the common factor to both lipid and carbohydrate hemostasis is the hormone insulin.

Reaven et al.¹⁶, compared a group of 41 survivors of myocardial infarction to an age matched control group for abnormalities of carbohydrate metabolism and hyperlipidemia. These studies demonstrated that 41% of the infarction group had abnormalities of oral glucose tolerance, whereas, only 2% of the control group showed such an abnormality.

Davidson and Albrink¹⁷, in studying patients with hypertriglyceridemia, have reported mild impairment of glucose tolerance and elevated plasma insulin responses.

Ostrander¹⁸, studied patients with the criteria for atherosclerotic heart disease and concluded that the proportion with elevated blood glucose levels and ab-

normal glucose tolerance tests was significantly greater than among persons of the same age and sex in the total population examined.

¹⁹
Kane et al. , in studying carbohydrate metabolism in 14 patients with hypertriglyceridemia, found abnormal glucose tolerance tests in 11, and noted decreased sensitivity to intravenously administered insulin during a glucose load. Free fatty acid levels were elevated and did not fall as rapidly or to as low levels as in normal subjects.

²⁰
Ahrens et al. , studied carbohydrate metabolism in patients with hypertriglyceridemia and suggested that patients with the carbohydrate-inducible forms might have defective secretion or utilization of insulin. He also commented that these individuals had responses to Tolbutamide resembling those seen in mild diabetes.

²¹
Farquhar et al. , studied 15 patients with hypertriglyceridemia without known diabetes mellitus. All had normal fasting blood sugars. In studying the triglyceride response to high carbohydrate feeding, they noted that all patients demonstrated increased endogenous triglyceride synthesis. The greatest degree of triglyceride response occurred in individuals with abnormal glucose

tolerance tests, and the higher accompanying rises in plasma insulin-like activity and immuno reactive insulin. They suggested that hyperglycemia in the presence of hyperinsulinemia resulted in hypertriglyceridemia.

²²
Reaven et al. , studied changes in triglyceride levels, and immuno reactive insulin in 33 hypertriglyceridemic patients during high carbohydrate feeding. All patients showed elevations of triglycerides in response to dietary carbohydrates. The magnitude of this response correlated proportionately to the degree of serum insulin response. It was postulated that increased insulin response in these patients was related to peripheral impairment of insulin and glucose uptake. As a response to peripheral insulin resistance, hyperinsulinemia would then develop and, in the presence of hyperglycemia, lead to an increase in the synthesis of endogenous triglycerides in the liver. Studies using liver slices from normal rats perfused with glucose following pre-treatment with insulin have shown that increased levels of insulin and glucose lead to increased lipogenesis in the liver.

²³
²⁴
Falsetti et al. , studied 27 patients with premature atherosclerotic heart disease for abnormalities of

carbohydrate and lipid metabolism. Eleven of 27 patients had abnormal glucose tolerance tests and 7 of the remaining 16 had abnormal cortisone glucose tolerance tests.

Seventeen of 27 had one or more abnormalities in serum lipids. They concluded that lipid abnormalities may be secondary to the decrease in glucose tolerance, as mediated by decreased removal or increased synthesis of lipid, or that the decrease in carbohydrate tolerance could be secondary to insulin resistance as a response to abnormal serum lipids. Tzagornis et al.²⁵, studied abnormalities of serum lipid levels, glucose tolerance and immuno reactive insulin concentrations. Abnormalities in one or more of these factors were demonstrated in 90% of these patients. Thirty had abnormally elevated serum lipids. They demonstrated that a significant degree of correlation exists between serum insulin and triglyceride levels. Treatment with Phenformin reduced triglyceride levels toward normal.

They concluded that carbohydrate and insulin abnormalities were frequent in young patients with coronary artery disease, and that hyperinsulinemia, in the presence of elevated glucose levels, gives rise to exaggerated production of triglycerides by the liver.

They also concluded that states in which an absolute or relative insulin deficiency exist may result in exaggerated free fatty acid mobilization in adipose tissue over and above the capacity of the liver to oxidize them with increased conversion to VLDL. Studies in vitro have demonstrated that exposure of arterial tissue to elevated levels of insulin, not only stimulates lipid synthesis, but also inhibits lipolytic activity²⁶. Both of these factors would encourage the^{27,} deposition of lipids in the intimal regions. Stout has shown that intravenous injection of rats with insulin and¹⁴ C-labelled substrate containing either glucose or acetate lead to a much greater incorporation of these substances into the aortic lipids than when the substrate alone was injected. He postulated that since insulin is known to inhibit tissue lipase in arterial tissue, excess of circulating insulin could cause accumulation of fat in the arterial wall by both increasing its deposition and inhibiting its removal. He suggested that insulin thus plays a major role in the pathogenesis of atherosclerosis.

C. CARBOHYDRATE INDUCTION STUDIES

The importance of dietary carbohydrate in the development of atherosclerosis has been alluded to above. Because of

the emphasis being placed upon the carbohydrate-inducible forms of hypertriglyceridemia and its association with a predisposition to the early development of coronary artery disease, methods of evaluating patients with the disorder are necessary.

In 1955, Hatch et al.²⁸, described increases of triglycerides and of S_f 20 - 400 lipoproteins in a study of hypertensives on a high carbohydrate, low fat, rice diet.

Since then, these results have been confirmed. Ahrens et al.²⁹, used formula feeding to differentiate the carbohydrate-inducible hypertriglyceridemia from the fat-induced form.

Characteristic plasma lipid pattern of the carbohydrate-inducible forms of hyperglyceridemia tend to show mild elevations of serum cholesterol with a predominant increase in the serum triglyceride levels. Lipoprotein electrophoresis on paper and agarose gel reveals a marked elevation in the pre-beta fraction. Similarly, analytical ultracentrifugation studies reveal increases in the S_f 20 - 400 fractions. This increase leads to the development of opalescence or turbidity of the plasma. Common features, in addition to the lipid changes stated above, consist of normal to elevated

fasting blood sugars, abnormalities in both the glucose tolerance and serum insulin levels after glucose administration. In spite of these abnormalities, the majority of these patients do not demonstrate clinical manifestations of diabetes mellitus.

³⁰
Kuo has demonstrated that high carbohydrate feeding in the form of sucrose can greatly exaggerate the degree of hyperglyceridemia in individuals with this abnormality, but that a very high (85 - 90%) carbohydrate diet is necessary to induce hypertriglyceridemia in normal individuals.

³¹
MacDonald , studied seven subjects on diets containing 500 gm of sucrose or raw maize starch for 25 days each and noted that on the former, serum triglyceride levels rose markedly, while on the latter they returned to normal or sub-normal values.

³²
Fredrickson and Lees , studied the effects of high carbohydrate feeding on normal males and females in an attempt to evaluate normal responses and amount of carbohydrate necessary to produce hypertriglyceridemia. In 12 of the 13 subjects, high carbohydrate feeding caused a rise in the plasma triglyceride. Triglycerides increased by 121 - 404 mg/100 ml (a mean rise of 224

mg/100 ml) from starting levels of 22 to 117 mg/100 ml (mean 62 mg/100 ml). Plasma turbidity, due to increased pre-beta lipoprotein, appeared within two to three days and usually reached a peak at 3 to 7 days. These authors concluded that about 7 gm/kg body weight of carbohydrate per day was necessary to elevate plasma triglyceride levels above 200 mg/100 ml. They judged the hyperlipemic response to high carbohydrate feeding to be abnormal only if plasma triglyceride was increased by more than 400 mg/100 ml over the baseline value, on a diet that contained 7 gm of carbohydrate/kg/day.

³³
Blankenhorn and Chin studied 15 young patients with known or suspected coronary artery disease. Ten of these were proved to have coronary artery disease angiographically. Of these 10, five were shown to have the carbohydrate-inducible form of hypertriglyceridemia. High carbohydrate feeding, in the amount of 7 gm/kg/day was administered for seven days to these ten patients; those with the Type IV abnormality showed rapid increases in triglyceride values in two to three days. In comparison, the remaining five patients without this type of abnormality showed no change or a slight fall in serum triglycerides.

D. THE PLASMA LIPOPROTEINS

The earliest concept of a lipid-protein relationship in blood was proposed by Nerking³⁴. Macheboeuf³⁵, in 1929, succeeded in isolating the first lipoprotein fraction of constant composition from horse serum. Gofman et al.³⁶, in 1949, proposed a method for the estimation of low density lipoproteins in the ultracentrifuge by using a medium sufficiently high in density that the lipoproteins would float forming optically discernible peaks which could be converted to units of concentration. For characterization, the floatation rates of various lipoprotein fractions were measured under standard conditions in a medium of density 1.063 gms/ml. The floatation rates were expressed in S_f units which are equivalent to negative Svedberg units of sedimentation. Protein concentrations decreased with increasing S_f values.³⁷ Lewis, Green and Page³⁷, identified high density lipoproteins by ultracentrifugation in a medium of density 1.21 gms/ml and presented the first correlation between ultracentrifugal and electrophoretic lipoprotein fractions. Gofman's techniques of analytical ultracentrifugation were and still are the primary standard for lipoprotein

analysis. Recent modifications and simplification of other analytical methods have allowed more practical methods of study. Techniques of protein chemistry and immunology have added the potential for recognition and quantitation of protein moieties to lipoprotein study.

The methods of lipoprotein analysis which lend themselves to practicability include zone electrophoresis (on paper and agarose), double diffusion immunological methods, immuno-electrophoresis, and combinations of preparative ultracentrifugation.

The major plasma lipids are cholesterol esters, phospholipids, triglycerides, free cholesterol and free fatty acids. Since none of these lipids possess sufficient polarity to circulate freely in solution, the lipid fractions combine with protein in complex macromolecules which have been classified according to size, density and electrophoretic mobility. The variability of ratios of protein and lipid to each other result in the variations in physical and chemical properties which form the basis for their study.

Two types of proteins are consistently isolated from plasma lipoproteins. In the lipid free state, these

are called apoproteins. They differ in amino acid content, terminal residues, and immuno - chemical behavior. Normally, the A protein is the only protein found in the alpha lipoprotein (HDL), and the B protein in the beta lipoprotein (LDL). Both proteins have been isolated in the VLDL and chylomicrons.

The A protein, when obtained after delipidation, appears to have a molecular weight of 23,000 to 36,000. It is believed that the A protein in native alpha lipoprotein represents a polymer of two to six sub-units. The A protein reacts with antisera against alpha lipoproteins. Estimates of the molecular weight of the alpha lipoprotein vary from 165,000 to 400,000. Light scattering techniques have indicated that the lipoprotein appears to be a prolate ellipsoid about 300 x 500 Angstroms³⁸. The B protein is more difficult to isolate. Estimates have indicated that its molecular weight varies between 250,000 and 380,000. The molecular weight of the beta lipoprotein has been estimated to be anywhere from $1.3 \times 3.2 \times 10^6$. Light scattering studies indicate that the usual complex is dyssymmetric and is in the form of a prolate ellipsoid of about 150 x 350 Angstroms.³⁹ The five main classes of lipoproteins which are isolated

in the plasma are chylomicrons, high density lipoproteins, low density lipoproteins, very low density proteins, and free fatty acids complexed with albumen;

i) Free fatty acids - albumen (FFA)

The unesterified FFA of plasma form a complex with albumen by non-covalent forces. In man, FFA metabolism provides about 50% of the energy requirements of the human being. These complexes are rapidly transported through plasma and have a half life of less than five minutes. This fraction, although part of the lipoprotein spectra, cannot be identified by the usual lipoprotein methods and must be measured by chemical or titrimetric methods.

ii) Alpha-Lipoproteins (HDL)

The high density lipoproteins (density 1.063 - 1.21 gms/ml) have been divided into three sub-fractions according to their behavior in the ultracentrifuge. They consist of approximately 50% protein, 30% phospholipid and smaller amounts of cholesterol, cholesterol esters and triglyceride. Tangier disease, an inherited deficiency of HDL, is associated with a low serum cholesterol and deposition of cholesterol esters in tissue.

iii) Beta-Lipoproteins (LDL)

The low density lipoproteins carry the great majority of lipids in the human plasma. About 50% by weight of LDL is cholesterol and its esters, while about 22% is protein. The fraction is isolated by preparative ultracentrifugation between densities of 1.006 and 1.019 gms/ml, and has an S_f value ranging between S_f 0 - 20. A-beta lipoproteinemia, a congenital absence of LDL, produces a syndrome of intestinal malabsorption and steatorrhea, neurological disorders, retardation and retinitis pigmentosa. Red blood cells show characteristic crenation which has been described as acanthocytosis. Serum triglycerides are very low, as is serum cholesterol. Levels of beta apoprotein have been shown to be normal, and the defect in the disease may be the inability to combine lipid with B protein. Elevated low density lipoproteins is the primary serum lipid abnormality seen in familial hypercholesterolemia. Environmental factors also affect low density lipoproteins, and this fraction is also modified by dietary cholesterol and fat intake.

iv) VLDL (pre-beta)

The principle lipid present in this fraction is triglyceride which, when present in large amounts, increases the particle size and decreases the density of the lipoprotein fraction. In the ultracentrifuge, this fraction is found between S_f 20 and 400, and may be isolated by adjusting the density to 1.006 gms/ml. Its principle function is transfer of triglyceride from liver to peripheral tissues. Increased levels may occur from increased biosynthesis in the liver or from impaired peripheral removal. Increased synthesis, in turn, may result from increased intake of fat, increased free fatty acid flux from adipose tissue, or diversion of intermediary compounds of carbohydrate metabolism. Impaired removal may result from defects in enzyme clearing mechanisms or structural defects in the lipoprotein. Increased levels of the fraction is associated with premature atherosclerosis.

v) Chylomicrons

The term chylomicron, originally defined particles present in plasma after the ingestion of a fatty meal. These particles are visible to light micr-

oscopy and consist of a fat emulsion stabilized by a membrane of protein, free cholesterol and phospholipids. The interior is primarily composed of triglycerides with small amounts of free and esterified cholesterol. Defective chylomicron removal occurs in hereditary disorders involving decreased activity of lipoprotein lipase.

E. LIPOPROTEIN ABNORMALITIES (HYPERLIPOPROTEINEMIAS)

In 1967, Fredrickson et al.⁴⁰, using an improved method of lipoprotein electrophoresis developed by Lees and Hatch⁴¹, described a system for phenotyping hyperlipoproteinemia. This system consisted of five major lipoprotein patterns, which were defined by paper electrophoresis.

Paper electrophoresis allows visualization of the kinds and amount of the soluble alpha and beta lipoproteins and separation of the glyceride-rich lipoproteins according to their sites of origin. Exogenous or dietary glyceride remains at the origin, whereas, endogenous glyceride forms a pre-beta band with an occasional trail of larger endogenous particles toward the origin. In normal subjects, the alpha and beta fractions are always visible while pre-beta lipoproteins become

detectable at concentrations of about 100 mg/100 ml. These five abnormalities have been associated with disease states such as nephrosis, myxedema, diabetes mellitus, pancreatitis, alcoholism, obstructive liver disease and dysproteinemias.

These phenotypes are demonstrated in figure 1⁴².

Type 1

This exogenous lipemia is characterized by the presence of chylomicrons in the plasma 14 to 16 hours after the last meal of a normal diet. Fasting plasma samples are creamy, the cholesterol is elevated and the triglyceride concentrations usually exceed 1000 mg/100 ml.

This rare type is believed to be inherited as a simple recessive trait. It is thought to be caused by a deficiency in post-heparin lipolytic activity (PHLA) as a result of deficiency in a serum enzyme lipoprotein lipase. Patients usually develop symptoms in early childhood and frequently show eruptive xanthomata, hepatosplenomegaly, lipemia retinalis, abdominal pain, and occasionally pancreatitis. A diagnosis requires the demonstration of fat intolerance and measurement of PHLA. Treatment consists of a fat restricted diet in which the total daily fat intake does not exceed

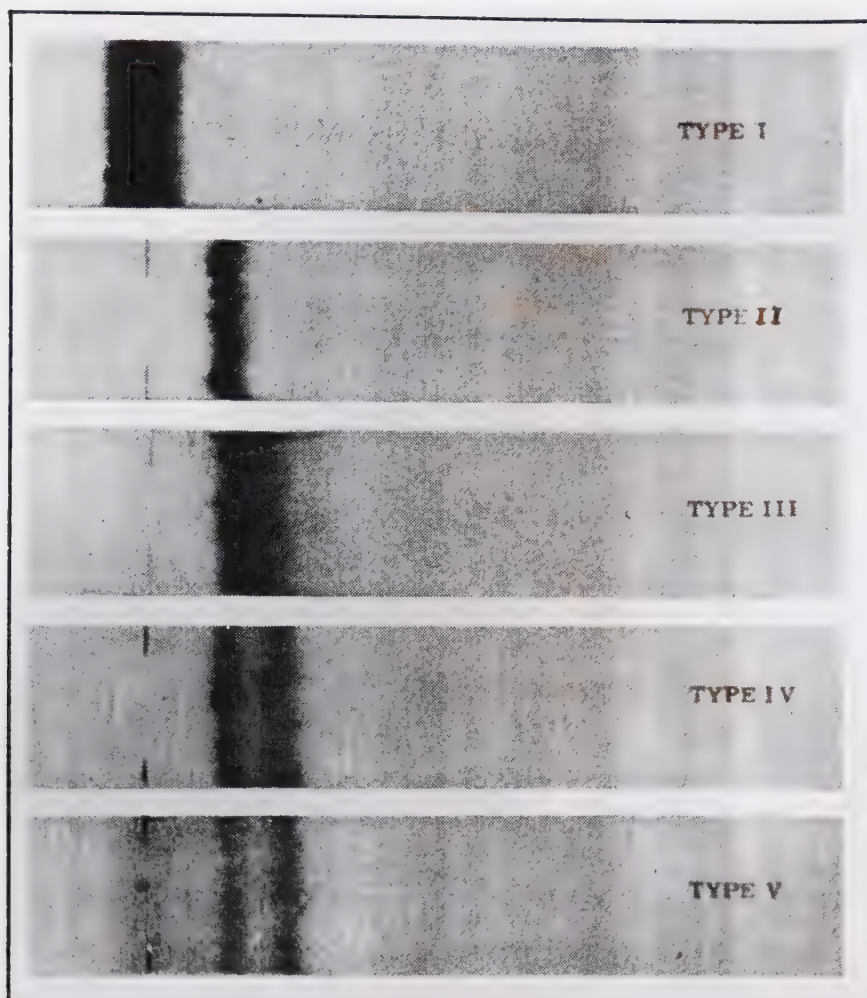


Figure 1: The Hyperlipoproteinemias (Types I - V)
by Paper Electrophoresis

25 to 50 gms per day.

Type II

This syndrome is characterized by an increased plasma concentration of beta lipoproteins. It is associated with severe and premature atheromatosis. Because of the solubility of the beta lipoprotein in plasma, patients present with marked hypercholesterolemia and clear plasma. It may arise from dietary excesses in cholesterol and saturated fat, while the familial form appears to be transmitted as a Mendelian dominant. Tendonous and tuberous xanthomata develop at an early age.

Treatment consists of a low cholesterol diet, the use of polyunsaturated fats, and in resistant cases, drug therapy. Cholestyramine is the agent of choice while moderate success has been achieved with the use of Atromid-S (clofibrate).

Type III

In type III, hyperlipoproteinemia, the beta lipoproteins are abnormally laden with glyceride. This lipid-laden beta lipoprotein is abnormal in composition and density. Confirmation of the disorder, also known as "Broad Beta Disease" requires the use of the preparative

ultracentrifuge. After ultracentrifugation of serum for 24 hours at a density of 1.006, the VLDL contain abnormal beta lipoproteins carrying a high content of glyceride. This is associated with an elevated cholesterol and triglyceride. Plasma may be clear or turbid depending on the concentration of the abnormal fraction. An almost specific clinical finding, in addition to xanthomatosis, is the presence of palmar xanthomata. Atherosclerosis is a common finding at an early age. About 40% have abnormal glucose tolerance tests, and many are sensitive to excess fat in the diet suggesting defects in exogenous and endogenous glyceride handling. Treatment includes restriction of fat in a manner similar to the Type II abnormality. Atromid-S has shown to be an effective agent in the management of this condition.

Type IV

The type IV hyperlipoproteinemia is perhaps the most common abnormality of lipoproteins in the American population. There is an increased concentration of the pre-beta fraction, reflecting an imbalance between synthesis and clearance of endogenous glycerides. Triglyceride values are increased but the serum chol-

esterol is normal or slightly elevated. On paper electrophoresis, the predominant fraction is the pre-beta band, and at high levels of triglyceride, this band tends to smear back toward the origin. The plasma may be clear, cloudy, or turbid depending on the level of triglycerides. Clinically, the patients present with premature atherosclerosis and with high triglyceride levels, xanthomatosis, lipemia retinalis, and hepatosplenomegaly. About 70 - 80% of these patients have mild to moderate abnormalities of glucose tolerance and about 60 - 70% respond with marked increases in triglyceride values with high carbohydrate feeding. Treatment consists of the maintenance of ideal weight, reduction of dietary carbohydrates to 150 gms/day, the use of unsaturated fats. Atromid-S has also been successful in lowering the elevated serum triglycerides.

Type V

The Type V hyperlipidemia is associated with the presence of both exogenous and endogenous glycerides in the plasma, with increased levels of serum cholesterol. Serum triglyceride values usually exceed 1,000 mg/100 ml, and the plasma is usually turbid or creamy. Paper electrophoresis reveals the presence of chylomicrons

and increased pre-beta lipoproteins. The inherited form is rare, and the condition occurs more commonly secondary to nephrosis, diabetes mellitus, pancreatitis, glycogen storage disease and other acute metabolic processes.

Eruptive xanthomas are common, as is lipemia retinalis and hepatosplenomegaly. Glucose intolerance is a common finding. PHLA is usually normal or only slightly reduced.

Treatment consists of a high protein diet with fat and carbohydrate restriction. Atromid-S has proven to be of considerable therapeutic value.

F. METHODS USED IN LIPOPROTEIN SEPARATION

The recognition of the importance of lipoproteins in the pathogenesis of many diseases including atherosclerosis has lead to the development of better quantitative and qualitative methods for elucidating the various fractions. These include electrophoresis, ultracentrifugation studies and immuno chemical techniques.

a) Electrophoresis

The development of electrophoretic techniques on fixed media has lead to the abandonment of lipoprotein studies employing free or moving boundary

electrophoresis. Today, these latter methods are of historical interest only.

Two of the major problems encountered in zonal electrophoresis are electroendosmotic buffer flow which proceeds in the opposite direction to lipoprotein migration and the steric and other interactions of lipoproteins with the electrophoretic media.

- i) Starch block electrophoresis - Starch block electrophoresis has been used extensively by Bierman⁴³, for the study of lipoproteins in abnormal metabolic states.
- ii) Paper - The earliest applications of paper electrophoresis to lipoprotein qualitation were carried out by Durrum⁴⁴. Improvements to these techniques were made by Smith⁴⁵, in 1957, with the addition of lipoprotein-poor serum to the buffer. However, the pre-beta fraction still showed considerable smearing over the beta fraction.
Lees and Hatch (1963)⁴¹, achieved good separation of the beta and pre-beta fractions with the addition of albumen to the buffer system.

- iii) Cellulose acetate - Chin and Blankenhorn⁴⁶, described a method of electrophoresis on cellulose acetate without the addition of albumen to the buffer. They demonstrated excellent resolution of all four lipoprotein classes. This method also afforded semi-quantitative evaluation of lipoprotein fractions by transmission microdensitometry.
- iv) Agarose gel - The striking feature of this method is the greater resolution of the VLDL from the beta lipoproteins.⁴⁷ Irwin and Campbell⁴⁷, described a method of agarose-gel electrophoresis which gives excellent resolution of all lipoprotein fractions, is reproducible and lends itself well to densitometric scanning.
- b) Ultracentrifugation
- The most complete and satisfactory method of quantitative evaluation of lipoprotein fractions is obtained by use of the analytical ultracentrifuge. This technique can be used to draw a continuous plot of concentrations of lipoproteins in floatation classes differing only by small increments.

This technique has been well described by Ewing⁴⁸
et al. .

The major drawback to this method is the cost of the apparatus and the inability of processing large numbers of samples quickly.

⁴⁹
Fredrickson, Levy and Lindgren , compared the interconvertibility of lipoprotein analyses obtained from electrophoretic techniques and analytical ultracentrifugation studies performed on pooled samples of hyperlipoproteinemias and lipoprotein deficiency states. A high degree of correlation existed between electrophoretic patterns and those obtained from the more quantitative ultracentrifugal studies. From these studies, the authors concluded that paper electrophoresis of lipoproteins is a useful, simple and rapid method for screening disorders of lipoprotein metabolism.

c) Immuno-Chemical Techniques

Immunological techniques have successfully been applied to the study of the structure and function of lipoproteins. They are extremely useful in the diagnosis of lipoprotein deficiency states. The two methods most commonly used are immuno-electrophor-

esis and gel diffusion techniques.

Quantitation of lipoproteins by immunological means⁵⁰
has been described by several authors .

G. CLOFIBRATE (p-CHLOROPHENOXYISOBUTYRATE)

The implication of plasma lipids as a primary or contributing factor in the pathogenesis of atherosclerosis has led to an intensive search for pharmacologic agents which will safely control the concentrations of lipids⁵¹ in the blood and tissues. Oliver , has laid down several criteria for safe, effective agents which lower serum lipids.

- i) They should have a sustained and consistent effect.
- ii) There should be no toxicity or side effects.
- iii) They should be easily administered.
- iv) Other serum lipids should be reduced.
- v) No accumulation of cholesterol, its precursors or its metabolites should occur in the plasma, reticulo-endothelial system, arteries or other tissues.
- vi) The mode of action should be known.

A substance which best satisfies the above criteria is ethyl- α -para-Chlorophenoxyisobutyrate (CPIB, Atromid-S, clofibrate).

⁵²
In 1962, Thorpe and Waring , studied a number of aryl-

oxyisobutyric acids in rats for their effect in lowering serum lipids. They found that the most suitable agent was para-chlorophenoxyisobutyric acid and its ethyl ester. Initially, they thought that clofibrate augmented a rhythmic endogenous hypocholesterolemic mechanism and suspected that androsterone might be the agent responsible. Oliver⁵³, and Hellman⁵⁴, demonstrated that a combination of clofibrate and androsterone, when administered orally, had a hypercholesterolemic and hypotriglyceridemic effect in man.

In early studies, Oliver⁵³, reported that clofibrate alone did not affect serum lipids. Hellman⁵⁴, later demonstrated that clofibrate alone possessed all the properties ascribed to the combination of clofibrate and androsterone.

Chemistry

Its empirical formula is $C_{12}H_{15}O_3Cl$. The molecular weight of clofibrate is 242.7 and its boiling point appears to be between 158 and 160 degrees Centigrade at 25 mm. It is stable, colorless to pale yellow, has a faint odor and characteristic taste, and is insoluble in water.

Absorption, Distribution and Excretion

The drug is absorbed in a linear manner from the gastro-

intestinal tract. After absorption, it undergoes rapid hydrolysis to the free acid which is extensively bound to the plasma proteins. Distribution appears to be limited to the plasma and extracellular fluid. In man, clofibrate is cleared from the plasma with an average half-life of 12 hours and appears in the urine as a glucouronide conjugate.

Effects on Lipids and Lipoproteins

The principle effect of clofibrate would appear to be on the triglyceride-rich lipoproteins VLDL (S_f 20 - 55 400) . It reduces the cholesterol-rich beta-lipoprotein (LDL) to a lesser degree. The drug appears to be effective in 75% of patients. The average reduction of cholesterol ranges from 7 to 35% (average 20%) and of triglycerides from 20 to 60% (average 35%). Phospholipids are also reduced. Coincident with a reduction in the low density lipoproteins is an increase in certain high density fractions.

Coagulation Effects

In early studies with this agent, it was found that a reduced dosage of anticoagulants was required when these drugs were used in combination with clofibrate⁵⁶ . Further studies revealed that there was a reduction

of fibrinogen levels⁵⁷ , and platelet stickiness⁵⁸ , and
prolongation of platelet survival time⁵⁹ .

Miscellaneous Effects

Transient elevations of SGOT and a slight decrease in alkaline phosphatase have been observed in a few patients within a few weeks of starting clofibrate.

Rats fed clofibrate have shown liver enlargement.

Liver cholesterol and triglycerides are reduced. Histologic examination of liver sections has shown an increased number of intracellular organelles, lysosomes and mitochondria. Clofibrate has been reported to reduce serum uric acid levels, particularly in patients with elevations of uric acid and the agent may have a
⁶⁰
primary uricosuric effect .

Mechanisms of Action

The precise mechanisms by which clofibrate reduces serum triglyceride and cholesterol is unknown.

⁶¹
Thorp , postulated that the agent displaced androsterone from plasma proteins in some unknown manner. These postulated hormonal effects have been subsequently disproved by studies on adrenalectomized and gonadectomized animals. In addition, clofibrate has been found to reduce lipids in thyroidectomized animals. Clofibrate

has been shown to effect a long-lasting inhibition of free fatty acid (FFA) release from adipose tissue with a modest, but significant, lowering of serum FFA levels.

In perfusion studies on isolated rat livers using ^{14}C -labelled palmitate, Duncan et al.⁶², found a 50% decrease in the secretion rate of triglyceride from the liver under the influence of clofibrate. In hyperglyceridemic human subjects, Spritz⁶³, found a marked shortening of the plasma $T_{1/2}$ for ^{14}C -labelled triglyceride when clofibrate was administered. Studies in rats⁶⁴, have suggested that clofibrate inhibits cholesterol biosynthesis in the liver at some step between acetate and mevalonate. There is no accumulation of demosterol or other cholesterol precursors. It has also been shown that the drug will enhance the excretion of bile acids and neutral sterols in the bile.

Contraindications

Definitive contraindications include patients with known or suspected liver or renal impairment. Pregnancy is also a relative contraindication.

Dosage

The daily adult dose recommended is 2 gm/day taken in

four divided doses.

Side effects

Adverse effects include urticaria, stomatitis, and pruritus. Weight gain has been reported in about 40% of patients. "Acute muscular syndromes", with elevations of lactic dehydrogenase, creatinine phosphokinase and aldolase have been reported by Langer and Levy⁶⁵. These have occurred, however, in patients on a dose of 4 gms/day and disappeared with the discontinuation of the drug.

Effects on Serum Insulin

⁶⁶
Zakim et al. , reported studies on a patient with carbohydrate-inducible hypertriglyceridemia. This patient demonstrated high fasting plasma insulin levels and an abnormal response to oral glucose at one hour which returned to normal after the administration of Atromid-S at a dose of 2 gm/day. Serum triglycerides also fell from 2400 mg/100 ml to 430 mg/100 ml. When Atromid-S was discontinued, plasma triglycerides rose rapidly, but the fasting insulin and the insulin response to oral glucose remained normal for several days. A glucose tolerance test during Atromid-S therapy was normal, but became diabetic in type fourteen days following the discontinuation of the drug.

From these studies, the authors postulated that the plasma insulin abnormality in patients with the carbohydrate-inducible form of hypertriglyceridemia was a secondary rather than a primary effect in this disorder.

METHODS AND PROCEDURES

A. METHODS

1. Paper Electrophoresis of Serum Lipoproteins⁴⁰

a) Equipment

Durrum-type electrophoretic cell, Spinco
Division, Beckman Instruments.

Regulated DC power supply.

Staining rack and tank.

Specimen applicator, Spinco Division,
Beckman Instruments.

20 lambda capillary tubes, Drummond Scientific.

b) Reagents

Barbital Buffer - Ionic strength 0.1, pH 8.6
containing 0.001 M EDTA and 1% human albumen
(Connaught).

To about 1200 ml of deionized water add the
contents of one bottle (24.5 gm) of LKB 3726-
VB Veronal (Barbital) buffer salts and mix.
Then add 15 gm of human albumen and 0.558 gm of
Disodium EDTA and mix gently on a magnetic
stirrer until the albumen is dissolved. Make
up to a volume of 1500 ml with deionized water.
With daily use, the buffer should be renewed
every four weeks.

Oil-Red-O-Stain - Add 0.6 gm Oil-Red-O dye to 500 ml of 60% ethanol (i.e. 950 ml 95% ethanol and 550 ml of deionized water). Heat to boiling, remove from heat and stir overnight. Place in a staining vessel without filtering, and keep at 37 - 40^o C. The stain must be made up every two weeks.

c) Procedure

Sample Collection:

The patient should be fasting for 12 - 14 hours before the sample is collected.

- i) Collect blood using EDTA as anticoagulant, centrifuge, and remove plasma.
- ii) Store plasma at 2 - 4^oC. Avoid freezing, as this will result in degradation of the lipoproteins.

Electrophoresis:

- i) Add 1 liter of barbital buffer, pH 8.6 to the electrophoretic cell.
- ii) Thoroughly moist strips and allow to equilibrate in the closed cell for 3 - 4 hours. Check that the buffer is the same level in both parts of the cell.

- iii) After equilibration, apply 20 lambda of serum to each strip. Apply seven specimens and one control serum.
 - iv) Carry out electrophoresis for 16 hours at a constant voltage of 110 volts with a current of .75 to 1.0 ma/strip.
 - v) After electrophoresis, dry the strips at 95° C for 20 minutes.
 - vi) Stain strips by immersion in the Oil-Red-O solution for 4 - 6 hours at 37 - 40° C.
 - vii) Rinse the strips in water for approximately 1 minute and air dry.
- d) Interpretation:
- Examine the strip for chylomicrons, beta, pre-beta and alpha bands. For proper interpretation, a plasma cholesterol and triglyceride should be determined on the specimen as well. A normal serum must be examined at the same time for comparison.

2. Agarose-gel Electrophoresis

a) Materials

"Sea-Kem" Agarose, Bausch and Lomb. Rochester, N.Y.

Buffer - LKB Veronal Buffer, ionic strength - 0.05, pH 8.6.

Dupont P 40B 35 mm safety motion picture film leader.

Model E800 Electrophoretic cell, Research Specialties Ltd., Berkeley, Calif.

Reservoir cutter.

Sudan Black B staining solution - 4 gm of Sudan Black B is added to 320 ml of absolute methanol, and mixed thoroughly. Eighty (80) ml of deionized water is added and the resulting solution is refluxed for approximately 10 minutes, then stored in a dark container at room temperature. The final staining solution consists of an 80% mixture of methanol - water saturated with Sudan Black B. Before use, the solution is filtered.

De-staining (rinsing) solution - Three hundred (300) ml of water is added to 700 ml of absolute methanol and allowed to cool to room temperature before use.

Anscomatic Film Developing Tank

Oster Airjet Hairdryer

b) Procedure

i) Preparation of agarose-gel strips

0.5 gm of agarose are added to 100 ml of LKB Veronal buffer and the mixture heated in a boiling bath for at least 1/2 hour. Following this, the solution is filtered by suction filtration into a previously warmed flask (80° C). The flask is then placed in the hot water bath to maintain the agarose preparation in solution until pouring. Approximately 33 ml of the above solution is poured on to the resin-treated side of 55 cm strips of leader tape and evenly spread. The poured strips are allowed to set without being disturbed for at least 45 minutes. Up to four of the above strips may be accommodated in the Model 800 Electrophoretic cell.

ii) Preparation of Samples and Electrophoresis

Five sample reservoirs (1mm x 15mm) are cut at 6 cm intervals along the agarose strip, the third reservoir placed in the center of the strip. The agarose remaining in

the reservoirs is carefully removed by a toothpick or a needle. The prepared agarose strip(s) is placed on the tap-water cooled (approximately 10°C) electrophoresis cell bed. Ten microliters of the desired plasma specimens are transferred to the respective reservoirs. Each specimen is run in duplicate. Constant voltage electrophoresis is performed for 30 minutes at 600 V (approximately 20 ma/strip). After electrophoresis is complete, the strip(s) are dehydrated in absolute methanol for 15 minutes. Following this, the agarose is dried by use of a hairdryer. The agarose remains as a thin film which adheres to the leader tape.

iii) Staining and De-staining

The dried strips are mounted on the reel and immersed in developing container containing 400 ml of previously prepared saturated Sudan Black B solution. Staining is carried out for exactly 30 minutes. After staining is complete, the reel cont-

aining the strips is removed and placed in the beaker containing enough 70% methanol in water solution to cover the reel. De-staining is carried out for 3 minutes, with de-staining solution (70% methanol-water) being replaced at 1 minute intervals. Following de-staining, the reel is immersed in deionized water for 30 seconds. This rinses off the de-staining solution, thus preventing further de-staining of the electropherograms. The strips are removed from the reel and air-dried at room temperature.

iv) Quantitation

The amount of lipid stained in the various lipoprotein fractions is measured by the use of densitometric scanning of the electropherograms. The area under each curve, representing a particular lipoprotein fraction, is obtained by the use of a suitable integrator. Three scanning instruments have been successfully used including CHROMOSCAN, GELSCAN AND DENSICORD.

A filter which transmits energy at a wavelength of 590 - 600 nanometers (nm) (blue) is used. A defining slit of approximately 1 x 15 mm gives good resolution and sensitivity. After obtaining the areas representing the lipoprotein fractions, the percent of each lipoprotein specimen presented is expressed as a percent of the total lipoprotein stained.

3. Serum Cholesterol

Serum cholesterol was measured by the semi-automated technique⁶⁷. An isopropanol extract of serum is reacted with a solution of sulfuric acid, glacial acetic acid and ferric chloride. Derivatives of perhydrocyclopentanophenanthrene having a 5-ene 3-ol grouping react with this reagent to produce a purple which can be measured at 550 nm. The analytical error of the method is ± 10 mg/100 ml (2 S.D.).

4. Serum Triglycerides

The semi-automated procedure for the determination of triglycerides is based on the work of Kessler and Lederer⁶⁸. Isopropanol extracts of serum are treated with a slurry containing zeolite, copper

lime and Lloyd's reagent. The lipid extract is then added to a base reagent automatically with saponification of triglyceride to glycerol in a 500° C heating bath. After saponification, periodate reagent is added to the reaction mixture to oxidize glycerol to formaldehyde. This is followed by condensation of formaldehyde with diacetylacetone and ammonia (Hantzsch Reaction) to give a fluorescent product, 3,5-diacetyl -1,4-dehydrolutidine. A 405 nm interference filter is used as the primary filter and a Corning 3-71 is used as the secondary filter. The analytical error of the method is ± 10 mg/100 ml (2 S.D.).

5. Glucose Tolerance Tests

Glucose tolerance tests were carried out following carbohydrate loading of all subjects for three days prior to the test. Following a fasting blood determination and the administration of 100 gm of glucose, blood sugars and urine determinations were obtained at half-hour intervals for 2½ hours.

Plasma glucose was measured by the potassium ferricyanide-potassium ferrocyanide reaction ⁶⁹.

Glucose reduces the yellow potassium ferricyanide

solution to colorless ferrocyanide. The reduction in color is proportional to the amount of glucose contained in the specimen and is measured at 420 nm in a colorimeter equipped with a flow cuvette which has a 15 mm light path. The analytical error of the method is ± 8 mg/100 ml (2 S.D.).

6. Immunoassay of Serum Insulin

Insulin immunoassays were carried out using the two antibody system of Morgan and Lazarow⁷⁰. This method is based on the fact that insulin reacts with anti-insulin antibody obtained from immunized guinea pigs (AIS-GP) to form a soluble complex. This soluble complex is precipitated by anti-guinea pig serum antibody obtained from immunized rabbits, (AGPS-R). When ¹³¹I-insulin is used as a tracer, the amount of radioisotope present in the precipitate is a function of the concentration of the insulin, unlabelled and labelled, present in the reaction mixture. The difference in percent of ¹³¹I-insulin precipitated ($\Delta\%$) when varying amounts of unlabelled insulin are added, forms the basis of the assay system.

7. Serum Free Fatty Acids

Determination of serum free fatty acid was carried out by the method of Trout, Estes and Friedburg⁷¹. This is an improved, more specific version of Dole's titrimetric method. The fatty acid solution that is to be titrated, is washed with 0.05% H_2SO_4 to remove lactic acid and an acetone-insoluble material that interferes with the titration. Heparinized samples for analysis were obtained at 15 minute intervals before and during intravenous infusion of glucose.

B. DIETARY STUDIES

1. Selection of Subjects

Twenty-one male patients with coronary artery disease between the ages of 35 and 49 (mean 43) were compared with 9 control subjects between 23 and 29 years of age (mean 25). Young patients were chosen because they might be expected to show certain lipoprotein abnormalities related to the development of atherosclerosis. Younger controls were selected to avoid the possibility of risk factors appearing in this group, thus making comparison difficult. The criteria for the diagnosis

of coronary artery disease included;

- i) definitive myocardial infarction confirmed by E.C.G. changes or serum enzyme changes or both (14 patients)
- ii) angina pectoris with angiographic evidence of coronary artery disease (5 patients)
- iii) angina pectoris associated with E.C.G. evidence of coronary ischemia (2 patients)

Since gross obesity is associated with abnormalities of glucose tolerance, serum lipids and lipoproteins, patients exceeding 115% of ideal body weight were excluded. Patients with myocardial infarction were not studied during the acute episode. Patients with documented diabetes mellitus were also excluded.

2. Diet

The high carbohydrate liquid diet was prepared in cooperation with the Metabolic Unit of the Department of Medicine, University of Alberta Hospital. The diet was composed of 85% carbohydrate (supplied as glucose) and 15% protein (supplied as skim milk powder). It contained 5 gm of NaCl per 1200 cc and was flavored according to individual taste with artificial flavorings. The administered amount

was calculated on the basis of body weight in kilograms, i.e. 30 cal/kg/body weight/day. Patients were instructed not to alter their diets prior to the administration of the test diet. It was consumed in equal amounts three times daily at mealtime. No other intake throughout the period of dietary administration was allowed except water. Dietary adjustments were made on a daily basis to maintain body weight within ± 1 kg/day.

3. Lipid and Carbohydrate Studies

Following hospital admission, baseline determinations were done on day four and day seven following completion of the dietary period.

C. CLOFIBRATE STUDIES

1. Selection of Subjects

Nine male hospital orderlies between 24 and 47 years of age were selected on the basis of absence of symptomatic coronary artery disease or known endocrine abnormalities.

2. Lipid, Carbohydrate and Insulin Studies

Baseline determinations included hemoglobin, white blood cell count, complete urinalysis, serum triglycerides, serum cholesterol, lipoprotein electrophoresis,

and glutamic oxaloacetic transaminase (SGOT). After a three day period of carbohydrate loading, 100 gm of glucose was administered orally in the fasting state with samples for plasma glucose and serum insulin drawn at 20 minute intervals for 2 hours. Clofibrate in a dose of 500 mg four times a day was then administered for 30 days and the above determinations repeated. There was a considerable variation in the plasma glucose values following oral glucose administration. Presumably this was due to variable absorption of glucose from the small bowel. Thus, it was decided to repeat the study using intravenous glucose infusion. Eight volunteers, 8 weeks following the cessation of previous clofibrate therapy were again studied in the baseline state in a similar manner to that outlined above. In this study, however, specimens were drawn at 15 minute intervals and, in addition, serum free fatty acids were also studied, before and after clofibrate administration for 14 days. Two subjects (P.K. and W.H.) were not available for this second study and one was replaced by another volunteer (P.K.). Glucose was infused

intravenously by a Harvard Pump at a rate of 500 mg/
min for 60 minutes.

RESULTS

A. THE EFFECT OF A SEVEN DAY HIGH CARBOHYDRATE DIET ON
SERUM LIPIDS IN NORMAL SUBJECTS AND PATIENTS WITH
CORONARY ARTERY DISEASE

A description of the control and patient groups taking part in the dietary study are shown in Table 1 and 2. The data in these tables include the age, clinical findings, and serum triglyceride and cholesterol values obtained throughout the seven day high carbohydrate diet. The mean age of the controls was 25 years with an age range of 23 - 29 years. The mean age of the patients was 43 years with an age range of 35 - 49 years. By the analytical methods employed in this study, the normal values for serum triglycerides and cholesterol in the age range of the control group are up to 140 mg/100 ml \pm 32 (2 S.D.), and up to 240 mg/100 ml \pm 41 (2 S.D.). Normal values of the above fractions in the age range of the patient group are up to 150 mg/100 ml \pm 37 (2 S.D.), and up to 250 mg/100 ml \pm 55 (2 S.D.)⁴⁰. Because of the gradual elevation of serum cholesterol that occurs with increasing age, the differences between the two groups shown in Table 4 are not felt to be highly significant. Serum triglycerides, however, increase very little with age and the results shown in Table 3 are felt to be highly significant.

TABLE 1

THE EFFECTS OF A SEVEN DAY HIGH CARBOHYDRATE DIET
ON THE SERUM LIPIDS OF CONTROL SUBJECTS

Pt.	Age (Yrs)	Triglycerides (mg/100 ml)			Cholesterol (mg/100 ml)		
		0	Day 4	7	0	Day 4	7
R.B.	28	95	150	175	200	147	150
P.K.	24	90	126	99	135	120	120
H.S.	29	75	105	210	168	145	135
J.O.	24	107	140	105	82	94	90
B.O.	24	83	85	68	160	105	100
T.S.	23	50	40	68	145	145	128
R.B.	23	125	70	85	165	134	128
R.D.	24	160	185	190	235	245	194
P.K.	28	215	285	255	225	180	128

TABLE 2

THE EFFECT OF SEVEN DAY HIGH CARBOHYDRATE DIET ON SERUM LIPIDS
OF YOUNG PATIENTS WITH CORONARY ARTERY DISEASE

Pt.	Age (Yrs)	Triglycerides (mg/100 ml)			Cholesterol (mg/100 ml)			Clinical Features & Age at Onset
		Day			Day			
		0	4	7	0	4	7	
P.H.	38	165	205	163	206	169	178	Acute Coronary Insufficiency 38 years
J.H.	37	180	350	240	245	105	182	Myocardial Infarction 36 years
F.H.	44	110	150	170	195	137	135	Myocardial Infarction 41 years
G.W.	42	215	210	245	180	187	165	Myocardial Infarction 42 years
H.S.	45	140	205	240	245	220	185	Myocardial Infarction 42 years
J.H.	35	125	200	170	352	304	275	Angina Pectoris 34 years
T.L.	40	170	205	262	440	282	290	Angina Pectoris 40 Years

TABLE 2 (CONTINUED)

THE EFFECT OF SEVEN DAY HIGH CARBOHYDRATE DIET ON THE SERUM
LIPIDS OF YOUNG PATIENTS WITH CORONARY ARTERY DISEASE

Pt.	Age (Yrs)	Triglycerides (mg/100 ml)			Cholesterol (mg/100 ml)			Clinical Features and Age at Onset
		Day			Day			
		0	4	7	0	4	7	
J.A.	49	220	475	645	370	270	283	Myocardial Infarction 46 years
D.A.	41	600	1225	950	220	185	175	Angina Pectoris 38 years
J.M.	40	274	390	480	253	225	200	Myocardial Infarction 38 years
H.H.	42	270	475	590	255	174	70	Myocardial Infarction 41 years
J.G.	35	480	450	390	290	295	270	Myocardial Infarction 34 years
E.M.	41	210	440	450	285	245	232	Myocardial Infarction 39 years
H.G.	44	600	930	925	285	235	230	Angina Pectoris 42 years

TABLE 2 (CONTINUED)

THE EFFECT OF SEVEN DAY HIGH CARBOHYDRATE DIET ON THE SERUM
LIPIDS OF YOUNG PATIENTS WITH CORONARY ARTERY DISEASE

Pt.	Age (Yrs)	Triglycerides (mg/100 ml)			Cholesterol (mg/100 ml)			Clinical Features and Age at Onset
		Day			Day			
		0	4	7	0	4	7	
E.B.	45	210	320	320	280	245	210	Myocardial Infarction 44 years
H.H.	39	228	320	378	285	185	130	Myocardial Infarction 38 years
H.T.	43	125	325	400	145	148	177	Myocardial Infarction 42 years
L.M.	40	200	450	500	290	205	190	Myocardial Infarction 37 years
N.M.	39	135	190	265	180	162	155	Myocardial Infarction 36 years
W.M.	48	190	450	450	235	159	149	Angina Pectoris 45 years
L.F.	40	275	375	450	190	210	240	Myocardial Infarction 38 years

1. Serum Triglycerides

Table 3 shows the range, mean and mean increase of serum triglycerides at zero, four and seven days for the control and patient groups on the high carbohydrate diet. The mean values of serum triglycerides (111, 132 and 145 mg/100 ml) for the control group were significantly lower ($p < 0.01$) than those of the patient group (244, 397 and 413 mg/100 ml). The mean increase of serum triglycerides, due to the high carbohydrate diet, was also much lower by day four and seven (21 and 34 mg/100 ml) in the control group ($p < 0.8$) than the mean increase (153 and 169 mg/100 ml) in the patient group ($p < 0.01$). Expressed as a percentage change, triglycerides of the control group increase by 19% and 30% on day four and seven as compared to 68% and 75% in the patient group. A graphical representation of the magnitude of change from day zero to day seven in the two groups studied is seen in Figure 2. The patient group has been subdivided into three groups; those with coronary artery disease and no lipoprotein abnormality; those with coronary artery disease and Type II hyperlipoproteinemia,

TABLE 3

AVERAGE EFFECT OF A SEVEN DAY HIGH CARBOHYDRATE DIET
ON SERUM TRIGLYCERIDES

Day	Range (mg/100 ml)		Mean (mg/100 ml)		Mean Increase (mg/100 ml)	
	Controls	Patients	Controls	Patients	Controls	Patients
0	50-215	110-600	111	224	-	-
4	40-285	150-1225	132	397	21	153
7	68-225	163-950	145	413	34	169

TABLE 4

AVERAGE EFFECT OF A SEVEN DAY HIGH CARBOHYDRATE DIET
ON SERUM CHOLESTEROL

Day	Range (mg/100 ml)		Mean (mg/100 ml)		Mean Decrease (mg/100 ml)	
	Controls	Patients	Controls	Patients	Controls	Patients
0	82-235	145-440	168	258	-	-
4	105-235	105-304	146	207	-22	-51
7	82-194	70-290	125	196	-43	-62

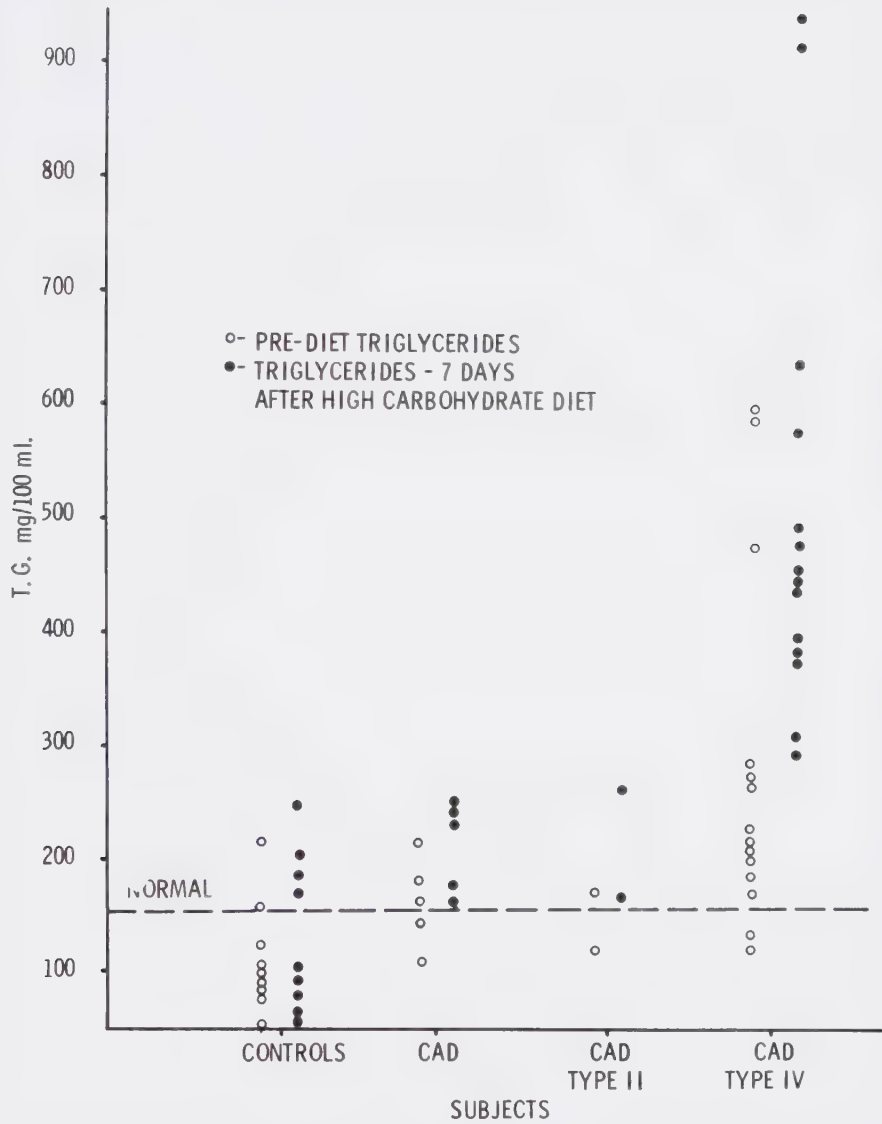


Figure 2: Effect of a High Carbohydrate Diet on the Serum Triglycerides (T.G.) of Controls and Patients with Coronary Artery Disease

and those with coronary artery disease and Type IV hyperlipoproteinemia. Graphically, it is noted that the greatest increase in serum triglycerides occurred in the Type IV patients after the seven day period of high carbohydrate feeding.

2. Serum Cholesterol

The range, mean and mean decrease of serum cholesterol at zero, four and seven days for the control and patient groups on the high carbohydrate diet is shown in Table 4. The mean values for serum cholesterol (168, 146 and 125 mg/100 ml) in the control group were significantly lower ($p < 0.01$) than those of the patient group (258, 207 and 196 mg/100 ml). The mean decrease of serum cholesterol on the high carbohydrate diet (-22 and -43 mg/100 ml) in the control group was less ($p < 0.05$) than the mean decrease (-51 and -62 mg/100 ml) in the patient group ($p < 0.01$). Expressed as a percentage change, serum cholesterol decreased by 13% and 26% on day four and seven as compared to 20% and 24% in the patient group. The magnitude of change in serum cholesterol levels in the groups studied is shown in Figure 3.

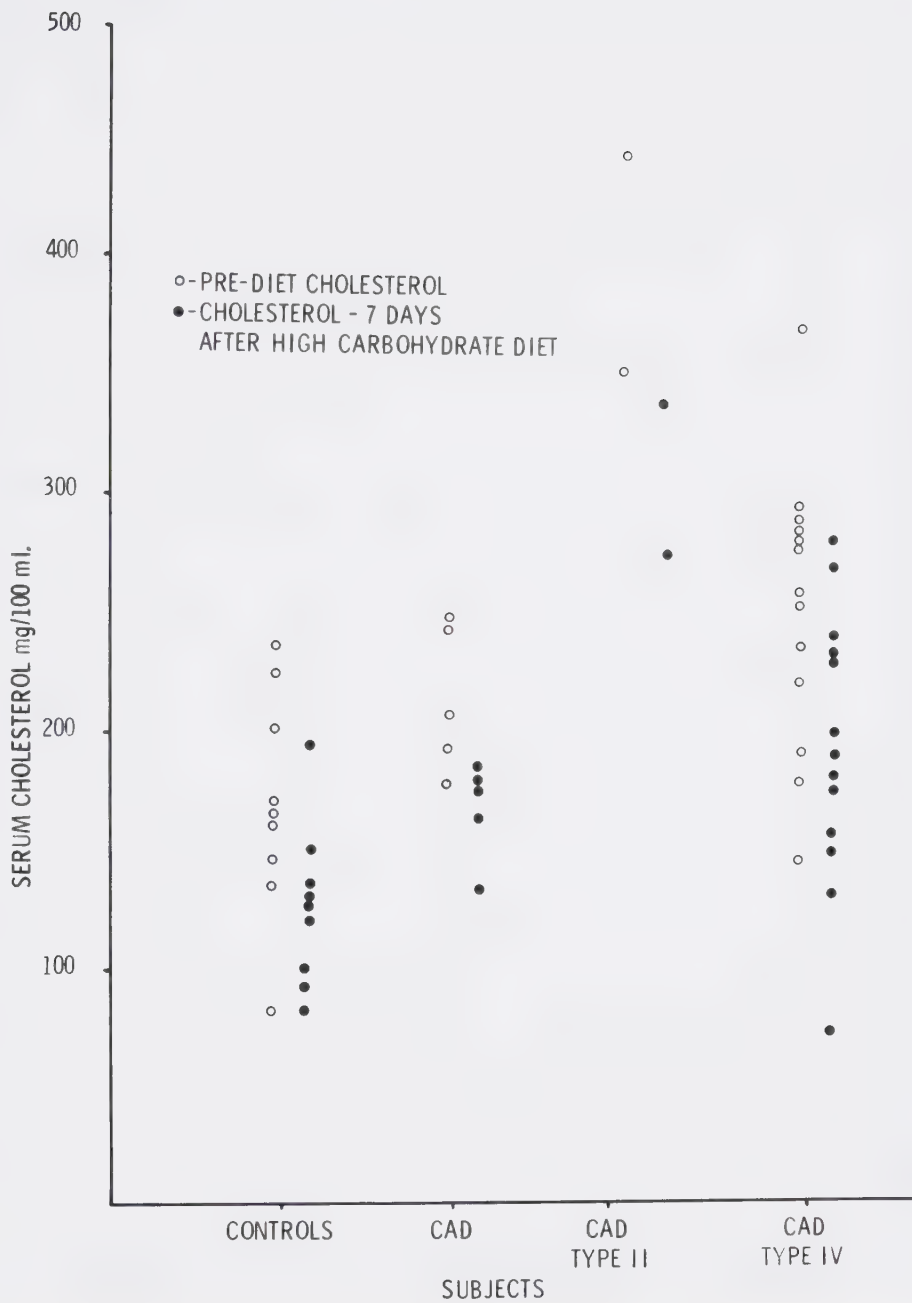


Figure 3: Effect of a High Carbohydrate Diet on the Serum Cholesterol of Controls and Patients with Coronary Artery Disease

3. Serum Lipoproteins

Serum lipoproteins were studied by paper and agarose gel electrophoresis. The results are seen in Tables 5 and 6.

By paper electrophoresis all control subjects had normal lipoprotein strips prior to the dietary study. Following the seven day high carbohydrate diet, 2 of 9 (2/9) normals demonstrated a slight increase in the pre-beta lipoprotein fraction.

Logically, both of these patients also showed the greatest increase in serum triglycerides.

Paper electrophoresis of the serum lipoproteins of the patient group prior to the dietary study revealed 11/21 to be normal, 2/21 showed prominent beta lipoprotein and 8/21 showed moderate to prominent pre-beta lipoprotein. At the completion of the dietary period, 6/11 of the previously normal strips showed prominent pre-beta lipoprotein as did the 8/21 which had shown this finding prior to the dietary challenge. Both patients with a prominent beta lipoprotein band showed moderate reductions of this fraction. The remaining 5/21 showed little or no significant change in serum

TABLE 5

THE EFFECT OF A SEVEN DAY HIGH CARBOHYDRATE DIET
ON THE SERUM LIPOPROTEINS OF CONTROL SUBJECTS

Pt.	Qualitative Paper Lipoprotein Evaluation		Qualitative Agarose Lipoprotein Evaluation	
	Day 0	Day 7	Day 0	Day 7
R.B.	-	slight elevation pre-beta	-	slight elevation pre-beta
P.K.	-	-	-	-
H.S.	-	slight elevation pre-beta	-	slight elevation pre-beta
J.O.	-	-	-	slight elevation pre-beta
B.O.	-	-	-	-
T.S.	-	-	-	-
R.B.	-	-	-	-
R.D.	-	-	slight elevation pre-beta	slight elevation pre-beta
P.K.	-	-	slight elevation pre-beta	slight elevation pre-beta

TABLE 6

THE EFFECT OF SEVEN DAY HIGH CARBOHYDRATE DIET ON SERUM
LIPOPROTEINS OF YOUNG PATIENTS WITH CORONARY ARTERY DISEASE

Pt.	Qualitative Paper Lipoprotein Evaluation		Qualitative Agarose Lipoprotein Evaluation		Glucose Tolerance Index
	Day 0	Day 7	Day 0	Day 7	
P.H.	-	-	-	slight elevation pre-beta	556
J.H.	-	-	-	slight elevation pre-beta	487
F.H.	-	-	-	slight elevation pre-beta	495
G.W.	-	-	-	slight elevation pre-beta	706
H.S.	-	-	-	slight elevation pre-beta	462
J.H.	elevated beta	elevated beta	elevated beta	slight elevation beta	365
T.L.	elevated beta	elevated beta	elevated beta	slight elevation beta	770

TABLE 6 (CONTINUED)

THE EFFECT OF SEVEN DAY HIGH CARBOHYDRATE DIET ON THE SERUM
LIPOPROTEINS OF YOUNG PATIENTS WITH CORONARY ARTERY DISEASE

Pt.	Qualitative Paper Lipoprotein Evaluation		Qualitative Agarose Lipoprotein Evaluation		Glucose Tolerance Index
J.A.	slight elevation pre-beta	marked elevation pre-beta	moderate elevation pre-beta	marked elevation pre-beta	493
D.A.	marked elevation pre-beta	very marked elevation pre-beta	marked elevation pre-beta	very marked elevation pre-beta	519
J.M.	elevated pre-beta	marked elevation pre-beta	moderate elevation pre-beta	marked elevation pre-beta	391
H.H.	elevated pre-beta	marked elevation pre-beta	moderate elevation pre-beta	marked elevation pre-beta	672
J.G.	marked elevation pre-beta	marked elevation pre-beta	marked elevation pre-beta	marked elevation pre-beta	466
E.M.	slight elevation pre-beta	marked elevation pre-beta	slight elevation pre-beta	marked elevation pre-beta	466
H.G.	marked elevation pre-beta	very marked elevation pre-beta	marked elevation pre-beta	very marked elevation pre-beta	514

TABLE 6 (CONTINUED)

THE EFFECT OF SEVEN DAY HIGH CARBOHYDRATE DIET ON THE SERUM
LIPOPROTEINS OF YOUNG PATIENTS WITH CORONARY ARTERY DISEASE

Pt.	Qualitative Paper Lipoprotein Evaluation		Qualitative Agarose Lipoprotein Evaluation		Glucose Tolerance Index
	Day 0	Day 7	Day 0	Day 7	
E.B.	-	elevated pre-beta	slight elevation pre-beta	moderate elevation pre-beta	787
H.H.	-	elevated pre-beta	slight elevation pre-beta	marked elevation pre-beta	533
H.T.	-	elevated pre-beta	-	marked elevation pre-beta	452
L.M.	-	elevated pre-beta	slight elevation pre-beta	marked elevation pre-beta	392
N.M.	-	elevated pre-beta	-	elevated pre-beta	446
W.M.	-	elevated pre-beta	slight elevation pre-beta	marked elevation pre-beta	466
L.F.	elevated pre-beta	elevation pre-beta	moderate elevation pre-beta	marked elevation pre-beta	441

lipoproteins at the completion of the study. In summary, after seven days of high carbohydrate feeding, the patient group in terms of lipoprotein abnormalities revealed 5/21 to be normal, 2/21 to demonstrate the Type II abnormality and 14/21 to demonstrate the Type IV abnormality. This latter group, logically demonstrated the greatest increase in serum triglycerides following the dietary challenge. One man (J.G.), although a classical Type IV on paper and agarose gel electrophoresis, failed to respond to high carbohydrate feeding with an increase in serum triglycerides.

By agarose-gel electrophoresis 2/9 subjects in the control group had slight, but detectable elevations of the pre-beta fraction prior to the period of dietary study. Following the dietary period, 5/9 demonstrated increases in the pre-beta fraction (Table 5). Two of these latter individuals had demonstrated elevations of pre-beta lipoprotein by the paper technique and showed the greatest elevations of serum triglycerides following the high carbohydrate diet. The remaining three also showed greater increases in serum triglycerides

than the remainder of the control group.

Examination of the agarose-gel electrophoretic strips in the patients done prior to the dietary challenge, revealed 7/21 to be normal, 2/21 to show prominent beta lipoprotein and 12/21 to show slight to marked elevations of the pre-beta fraction. At the completion of the dietary period, 5/7 of the patients who appeared normal prior to the administration of the diet developed slight increases in pre-beta lipoproteins, but were felt to be qualitatively normal. The remaining 2/7 who had appeared normal prior to the diet, developed significantly elevated pre-beta lipoprotein. Both patients with prominent beta lipoprotein showed moderate reductions in this fraction. The remaining 11/21 patients demonstrated moderate to marked increases in the pre-beta lipoprotein fraction comparable to the Type IV abnormality.

In Figure 4 is shown serum lipoproteins separated by paper electrophoresis of a normal and a Type II individual. The increase of beta lipoprotein is noted in the Type II serum.

In Figure 5 is shown the serum lipoproteins of a

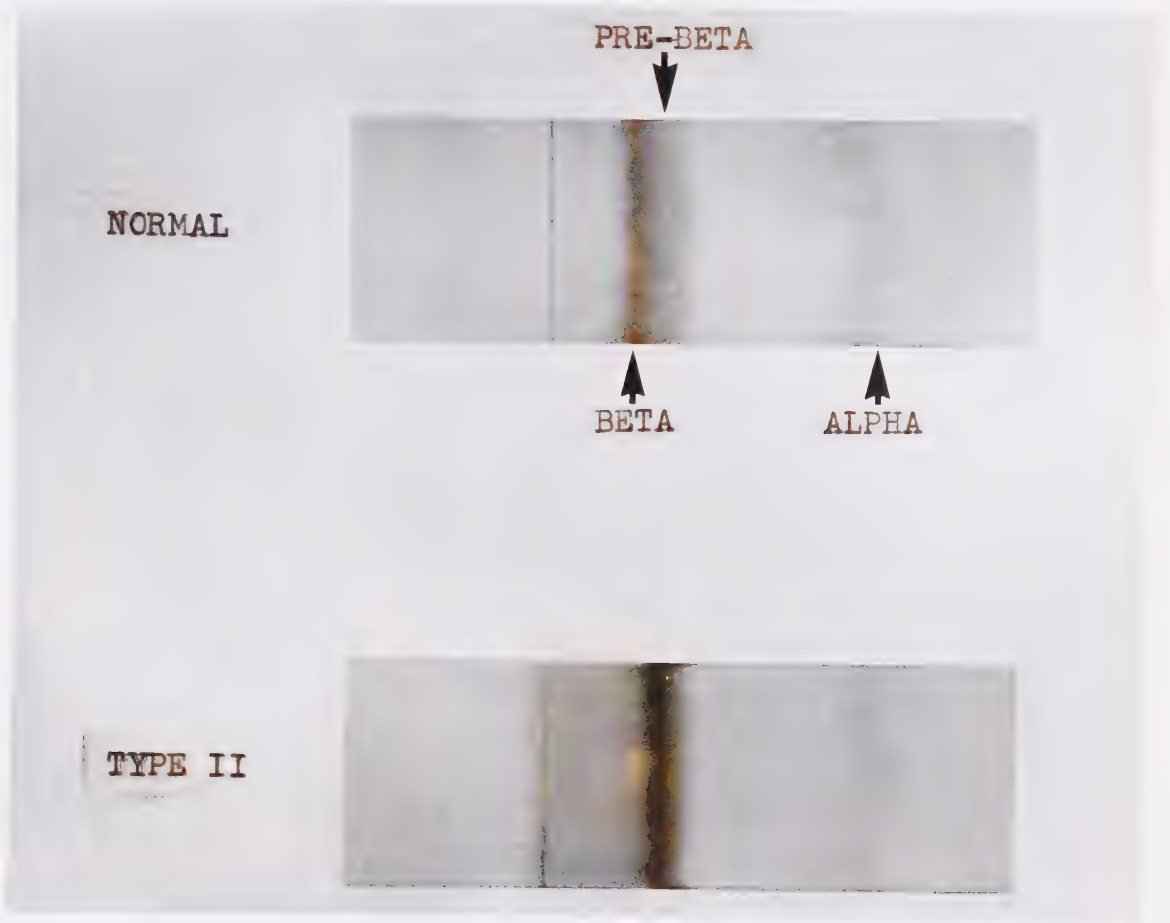


Figure 4: Normal and Type II Hyperlipoproteinemia by
Paper Electrophoresis

TYPE IV
BEFORE DIET



TYPE IV
AFTER DIET



Figure 5: Type IV Hyperlipoproteinemia Before and After
Seven Days of a High Carbohydrate Diet

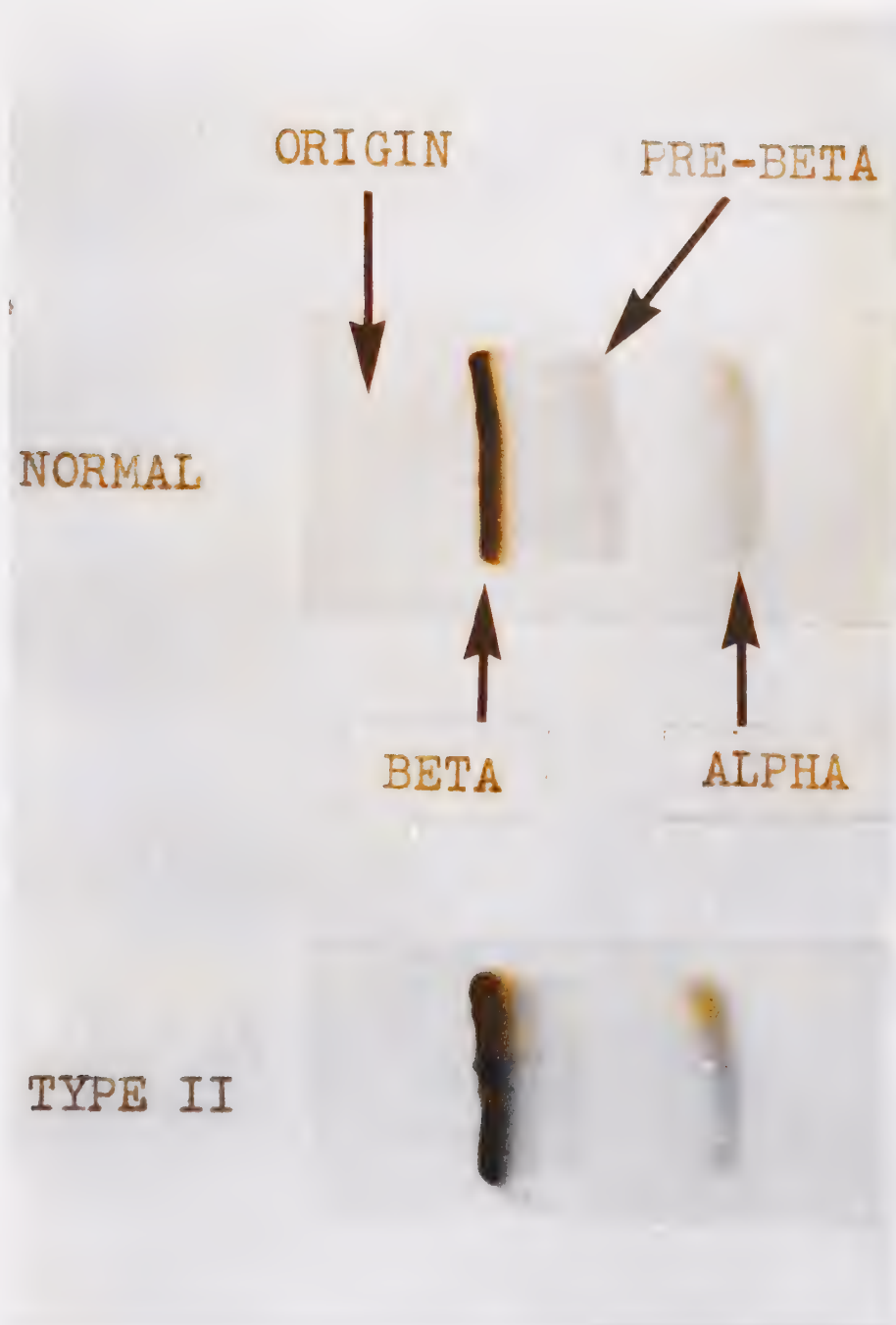
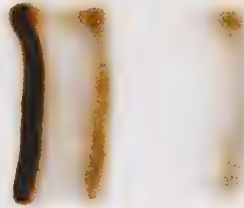


Figure 6: Normal and Type II Hyperlipoproteinemia by
Agarose Gel Electrophoresis.

TYPE IV

BEFORE DIET



TYPE IV

AFTER DIET



Figure 7: Type IV Hyperlipoproteinemia by Agarose Gel Electrophoresis Before and After Seven Days of a High Carbohydrate Diet.

Type IV individual before and after seven days of high carbohydrate feeding. The increase in the pre-beta fraction is well demonstrated.

In Figure 6, is shown serum lipoproteins separated by agarose gel electrophoresis of a normal and Type II individual. The increase of beta lipoproteins is noted in the Type II serum.

In Figure 7, is shown the serum lipoproteins of a Type IV individual before and after seven days of high carbohydrate feeding. The marked increase in the pre-beta fraction is well demonstrated.

4. The Effect of a Seven Day Carbohydrate Diet on Serum Lipoprotein Fractions as Separated by Agarose - Gel Electrophoresis and Quantitated by Densitometric Scanning.

The changes in relative percentages of the lipoprotein fractions in the control and patient groups following seven days of high carbohydrate feeding are shown in Tables 7 and 8. These are values of beta, pre-beta and alpha lipoproteins obtained from agarose strips and expressed as a percentage of the total lipid stained. The range, mean and mean change (increase or decrease) of the relative per-

TABLE 7

EFFECT OF A SEVEN DAY HIGH CARBOHYDRATE DIET ON THE
SERUM LIPOPROTEIN FRACTIONS OF CONTROLS AS SEPARATED BY
AGAROSE-GEL ELECTROPHORESIS AND QUANTITATED
BY DENSITOMETRIC SCANNING

Pt.	RELATIVE PERCENTAGE OF LIPOPROTEIN FRACTION					
	beta		pre-beta		alpha	
	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
R.B.	64	53	11	28	25	19
P.K.	50	50	17	15	33	35
H.S.	62	34	10	51	28	15
J.O.	46	37	15	28	39	35
B.O.	57	48	14	13	29	39
T.S.	44	44	5	5	51	51
R.B.	53	31	16	39	31	30
R.D.	67	57	15	29	18	14
P.K.	55	33	31	50	14	17

TABLE 8

EFFECT OF SEVEN DAY HIGH CARBOHYDRATE DIET ON THE SERUM
LIPOPROTEIN FRACTIONS OF YOUNG PATIENTS WITH CORONARY ARTERY
DISEASE AS SEPARTED BY AGAROSE-GEL ELECTROPHORESIS AND QUANT-
ITATED BY DENSITOMETRIC SCANNING

Pt.	RELATIVE PERCENTAGE OF LIPOPROTEIN FRACTION					
	beta		pre-beta		alpha	
	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
P.H.	53	47	30	35	17	18
J.H.	54	41	29	44	17	15
F.H.	45	44	27	33	28	23
G.W.	67	36	20	60	13	4
H.S.	74	52	4	38	22	10
J.H.	80	65	7	24	13	11
T.L.	78	67	11	24	11	9

TABLE 8 (CONTINUED)

EFFECT OF A SEVEN DAY HIGH CARBOHYDRATE DIET ON THE SERUM
LIPOPROTEIN FRACTIONS OF YOUNG PATIENTS WITH CORONARY ARTERY
DISEASE AS SPEARTED BY AGAROSE GEL ELECTROPHORESIS AND QUAN-
TITATED BY DENSITOMETRIC SCANNING

Pt.	RELATIVE PERCENTAGE OF LIPOPROTEIN FRACTION					
	beta		pre-beta		alpha	
	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
E.B.	74	55	17	36	9	9
H.H.	66	34	22	55	12	11
H.T.	75	25	0	39	25	14
L.M.	55	61	37	26	8	10
N.M.	60	35	15	51	25	14
W.M.	60	24	26	69	14	7
L.F.	46	41	38	46	16	13

TABLE 8 (CONTINUED)

EFFECT OF A SEVEN DAY HIGH CARBOHYDRATE DIET ON THE SERUM
LIPOPROTEIN FRACTIONS OF YOUNG PATIENTS WITH CORONARY ARTERY
DISEASE AS SEPARATED BY AGAROSE GEL ELECTROPHORESIS AND
DENSITOMETRIC SCANNING

Pt.	RELATIVE PERCENTAGE OF LIPOPROTEIN FRACTION					
	beta		pre-beta		alpha	
	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
J.A.	25	12	65	84	10	4
D.A.	25	8	70	89	5	8
J.M.	56	29	40	69	4	2
H.H.	56	18	25	78	19	4
J.G.	51	53	43	42	6	5
E.M.	60	61	20	20	20	19
H.G.	38	15	57	81	5	5

centages of the lipoprotein fractions are shown in Tables 9,10 and 11.

The mean values of pre-beta for the control group at day zero and seven (15% and 29%) were significantly lower ($p < 0.01$) than the patient group (28% and 50%). The mean increase in the control group (14% versus 22%) was smaller numerically than the patient group, but not statistically significant.

The mean values of beta lipoprotein for the control group at day zero and seven (55% and 43%) were not significantly lower ($p < 0.7$) than the patient group (57% and 40%). The mean decrease in the control group (12%) was not significantly different than in the patient group (17%).

The mean values of alpha lipoprotein for the control group at day zero and seven (30% and 28%) were significantly higher ($p < 0.01$) than the patient group (14% and 10%). The mean decrease in the control group (2%) was not significantly different than in the patient group (4%).

TABLE 9

CHANGES IN THE RATIO OF PRE-BETA TO OTHER LIPOPROTEIN

FRACTIONS DUE TO HIGH CARBOHYDRATE DIET

Day	Range of % Pre-beta		Mean of % Pre-beta		Increase of Mean % Pre-beta	
	Controls	Patients	Controls	Patients	Controls	Patients
0	5 - 31	0 - 65	15	28	-	-
7	5 - 50	20 - 89	29	50	14	22

TABLE 10

CHANGES IN THE RATIO OF BETA TO OTHER LIPOPROTEIN

FRACTIONS DUE TO HIGH CARBOHYDRATE DIET

Day	Range of % Beta		Mean of % Beta		Decrease of Mean % Beta	
	Controls	Patients	Controls	Patients	Controls	Patients
0	46 - 67	25 - 88	55	57	-	-
7	31 - 57	8 - 67	43	40	-12	-17

TABLE 11

CHANGES IN THE RATIO OF ALPHA TO OTHER LIPOPROTEIN

FRACTIONS DUE TO HIGH CARBOHYDRATE DIET

Day	Range of % Alpha		Mean of % Alpha		Decrease of Mean % Alpha	
	Controls	Patients	Controls	Patients	Controls	Patients
0	14 - 51	5 - 28	30	14	-	-
7	14 - 51	2 - 23	28	10	-2	-4

5. Glucose Tolerance Tests in Patients with Premature Coronary Artery Disease.

The sum of the fasting, one hour, one and one-half hour and two hour plasma glucose values for each member of the patient group was expressed as the "glucose tolerance index"⁷² (Table 6). This numerical value was selected as the best single expression of both the peak and duration of the glucose tolerance curve. Normal values of plasma glucose should not exceed the following;

Fasting plasma glucose-----115 mg/100 ml

One hour plasma glucose-----185 mg/100 ml

One and one half-hour plasma
glucose -----155 mg/100 ml

Two hour plasma glucose-----125 mg/100 ml

The sum of these values is 580 and normal values should not exceed this level.

In the patient group 4/21 had a glucose tolerance index which exceeded 580. Two occurred in the Type IV patients (787, 672), one in the Type II patients (770) and one in the group without demonstrable lipoprotein abnormalities (706) (Figure 8).

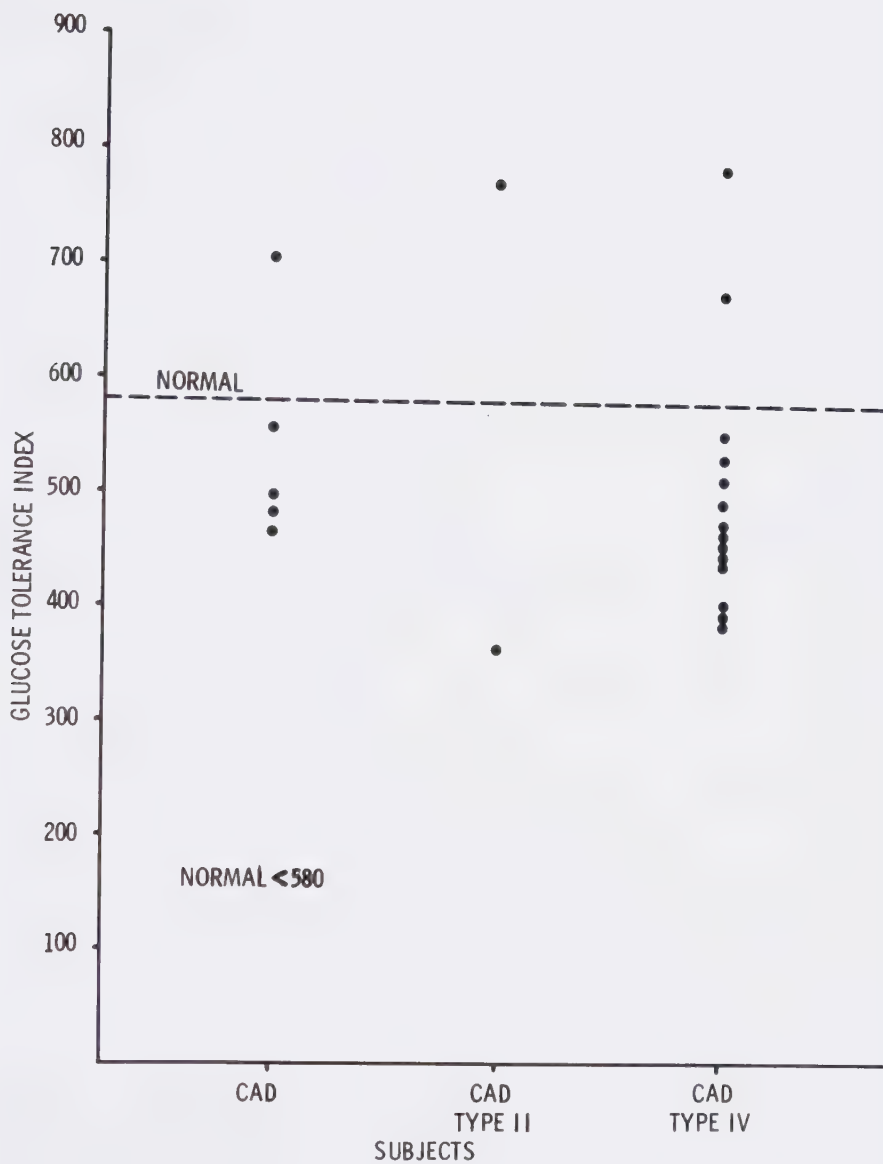


Figure 8: Glucose Tolerance Index of Patients with Coronary Artery Disease

B. THE EFFECTS OF CLOFIBRATE IN NORMAL SUBJECTS

A description of the normal subjects taking part in the clofibrate study is shown in Table 12. The data in this table shows the age, serum triglyceride and cholesterol values and lipoprotein findings before and after clofibrate administration. The mean age of the group was 41 years with an age range of 29 - 49 years.

1. Serum Triglycerides

Table 13 shows the range, mean and mean decrease of serum triglyceride values of the experimental group before and at the completion of 30 days of drug administration. The mean value for serum triglycerides prior to clofibrate therapy was 221 mg/100 ml and fell to 112 mg/100 ml at the completion of the study. This is a mean decrease of 109 mg/100 ml (49%) but because of the small number of subjects in the group and the inclusion of a subject (A.M.) with marked elevation of serum triglycerides in comparison with other subjects, the change is not statistically significant ($p < 0.2$). The elevation of serum triglycerides in this subject imparts a large standard deviation to this parameter, \pm 190 mg/100 ml before drug administration

TABLE 12

THE EFFECTS OF THIRTY DAYS OF CLOFIBRATE
ON THE SERUM LIPIDS OF NORMAL SUBJECTS

Pt.	Age (Yrs)	Triglycerides (mg/100 ml)		Cholesterol (mg/100 ml)	
		Day 0	Day 30	Day 0	Day 30
P.G.	48	185	107	145	150
G.V.	36	190	112	240	170
D.A.	41	145	72	212	157
P.K.	46	60	50	215	170
A.B.	34	110	120	200	140
H.G.	48	230	125	130	145
A.M.	36	700	237	185	195
N.A.	49	270	120	140	147
W.H.	29	105	70	130	103

TABLE 13

AVERAGE EFFECT OF THIRTY DAYS OF CLOFIBRATE ON
THE SERUM TRIGLYCERIDES OF NORMAL SUBJECTS

Day	Range (mg/100 ml)	Mean (mg/100 ml)	Mean Decrease (mg/100 ml)
0	60 - 700	221	-
30	50 - 237	112	-109

TABLE 14

AVERAGE EFFECT OF THIRTY DAYS OF CLOFIBRATE ON
THE SERUM CHOLESTEROL OF NORMAL SUBJECTS

Day	Range (mg/100 ml)	Mean (mg/100 ml)	Mean Decrease (mg/100 ml)
0	130 - 240	177	-
30	103 - 195	153	-24

and \pm 53 mg/100 ml after. By excluding this man, the mean value for serum triglycerides prior to clofibrate was 162 mg/100 ml and fell to 97 mg/100 ml with a mean decrease of 65 mg/100 ml (40%). This reduction is statistically significant ($p < 0.05$).

2. Serum Cholesterol

Table 14 shows the range, mean and mean decrease of serum cholesterol values of the experimental group before and after completion of drug administration. The mean value for serum cholesterol prior to clofibrate therapy was 177 mg/100 ml and fell to 153 mg/100 ml at the completion of the study. This is a mean decrease of 24 mg/100 ml (13%), but is not statistically significant ($p < 0.2$).

3. Serum Lipoproteins

These were studied by both paper and agarose-gel electrophoresis.

By paper electrophoresis, 7/9 subjects had normal lipoproteins prior to drug administration (Table 15). Two of nine had elevated pre-beta lipoproteins, one man (A.M.) to a marked degree. At the end of 30 days of clofibrate, 8/9 were normal with only

TABLE 15

THE EFFECTS OF THIRTY DAYS OF CLOFIBRATE ON

THE SERUM LIPOPROTEINS OF NORMAL SUBJECTS

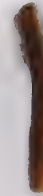
Pt.	Qualitative Paper Lipoprotein Evaluation		Qualitative Agarose Lipoprotein Evaluation	
	Day 0	Day 30	Day 0	Day 30
P. G.	-	-	-	reduced pre-beta
G. V.	-	-	-	reduced pre-beta
D. A.	-	-	-	reduced pre-beta
P. K.	-	-	-	reduced pre-beta
A. B.	-	-	-	reduced pre-beta
H. G.	slight elevation pre-beta	-	slight elevation pre-beta	-
A. M.	marked elevation pre-beta	elevated pre-beta	very marked elevation pre-beta	moderate elevation pre-beta
N. A.	-	-	slight elevation pre-beta	-
W. H.	-	-	-	-

one man (A.M.) showing a prominent, but reduced, pre-beta fraction in comparison to his previous strip.

When studied by agarose-gel electrophoresis, 6/9 had normal lipoproteins prior to drug administration (Table 15). Three of nine had elevations of the pre-beta lipoproteins, including A.M., who had also shown a marked elevation in this fraction by paper electrophoresis. Following clofibrate administration, 8/9 showed reduced or absent pre-beta lipoproteins by this technique. One subject, (A.M.) showed marked reduction of the pre-beta fraction from the baseline state, although it was still elevated as compared to normal. Three of nine showed a complete absence of pre-beta lipoprotein when studied by this technique.

In Figure 9 is shown the serum lipoproteins separated by agarose-gel electrophoresis of a normal individual before and after 30 days of clofibrate therapy. The marked reduction in the pre-beta fraction is well-demonstrated.

NORMAL
BEFORE CLOFIBRATE



NORMAL
AFTER CLOFIBRATE



Figure 9: Effect of 30 Days of Clofibrate on Serum Lipoproteins Separated by Agarose Gel Electrophoresis

4. Changes in Lipoprotein Fractions as Revealed by Densitometric Scanning of Agarose Gel Electropherograms

In Table 16 are detailed the changes in relative percentages of the subjects' lipoprotein fractions following 30 days of clofibrate ingestion. These are the values of pre-beta, beta and alpha lipoproteins obtained from agarose strips expressed as a percentage of the total lipoproteins stained. The range, mean and mean change (increase or decrease) of the relative percentages of each lipoprotein fraction is shown in Tables 17, 18 and 19.

The mean value of pre-beta lipoprotein on day zero (32%) was higher than on day thirty (14%) but this decrease was not highly significant ($p < 0.02$).

The mean value of beta lipoprotein on day zero (46%) was lower than on day thirty (61%) but not highly significant ($p < 0.02$).

The mean value of alpha lipoprotein on day zero (22%) was lower than on day thirty (25%) but not significant ($p < 0.4$).

TABLE 16
EFFECT OF THIRTY DAYS OF CLOFIBRATE ON SERUM LIPOPROTEIN
FRACTIONS AS SEPARATED BY AGAROSE GEL
ELECTROPHORESIS AND QUANTITATED BY DENSITOMETRIC SCANNING

Pt.	Relative Percentage Changes of Lipoprotein Fractions					
	beta		pre-beta		alpha	
	Day 0	Day 30	Day 0	Day 30	Day 0	Day 30
P.G.	28	46	46	19	26	35
G.V.	55	61	29	15	16	24
D.A.	53	68	25	6	22	26
P.K.	58	66	9	0	33	34
A.B.	55	58	24	18	21	24
H.G.	40	70	39	12	21	18
A.M.	26	47	66	42	18	11
N.A.	50	69	29	11	21	20
W.H.	53	64	19	0	28	36

TABLE 17

CHANGES IN THE RATIO OF PRE-BETA TO OTHER LIPOPROTEIN

FRACTIONS AFTER THIRTY DAYS OF CLOFIBRATE

Day	Range of % Pre-beta Present	Mean of % Pre-beta Present	Decrease of Mean % of Pre-beta Present
0	6 - 66	32	-
30	0 - 42	14	18

TABLE 18

CHANGES IN THE RATIO OF BETA TO OTHER LIPOPROTEIN

FRACTIONS AFTER THIRTY DAYS OF CLOFIBRATE

Day	Range of % Beta Present	Mean of % Beta Present	Increase of Mean % of Beta Present
0	26 - 58	46	-
30	46 - 59	61	15

TABLE 19

CHANGES IN THE RATIO OF ALPHA TO OTHER LIPOPROTEIN

FRACTIONS AFTER THIRTY DAYS OF CLOFIBRATE

Day	Range of % Alpha Present	Mean of % Alpha Present	Increase of Mean % of Alpha Present
0	8 - 28	22	-
30	11 - 36	25	3

5. The Effect of Thirty Days of Clofibrate Administration on Plasma Glucose Levels During Oral Glucose Challenge

Table 20 and Figure 10 show the plasma glucose values at 20 minute intervals on day zero and thirty following the administration of 100 gm of glucose orally. After one month of clofibrate administration, plasma glucose values tended to be higher at 60, 80, 100 and 120 minutes. However, this is not a statistically significant change.

6. The Effect of Thirty Days of Clofibrate Administration on Serum Insulin Levels During Oral Glucose Challenge

Table 20 and Figure 11 show the serum insulin levels in $\mu\text{u/ml}$ (micro units/ml) at 20 minute intervals on day zero and thirty following the administration of 100 gm of glucose orally. After one month of clofibrate administration, serum insulin values rose to lower levels at 20 and 40 minutes and increased levels at 60 and 80 minutes. These differences, however, are not statistically significant.

TABLE 20

AVERAGE EFFECT OF THIRTY DAYS OF CLOFIBRATE ADMINISTRATION

ON PLASMA GLUCOSE AND SERUM INSULIN LEVELS

DURING ORAL GLUCOSE CHALLENGE

Time (min)	Plasma Glucose mg/100 ml ± 2 S.D.		Serum Insulin mu/ml ± 2 S.D.	
	Day 0	Day 30	Day 0	Day 30
-20	88 ± 12	87 ± 16	24 ± 22	27 ± 34
0	89 ± 5	86 ± 9	37 ± 68	27 ± 30
20	125 ± 23	103 ± 29	101 ± 126	60 ± 64
40	158 ± 35	154 ± 68	133 ± 118	107 ± 94
60	162 ± 58	176 ± 76	127 ± 62	142 ± 128
80	143 ± 52	175 ± 88	126 ± 110	158 ± 82
100	128 ± 64	155 ± 94	122 ± 116	120 ± 118
120	117 ± 60	143 ± 66	117 ± 150	108 ± 86

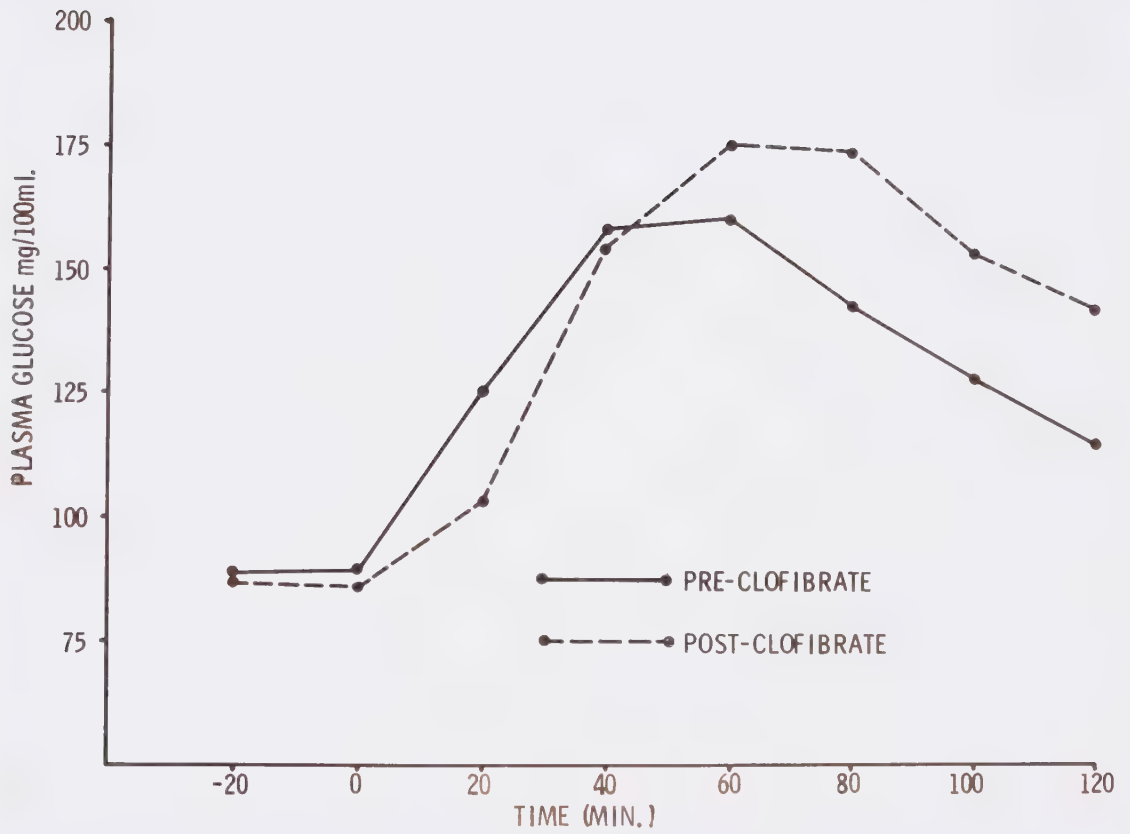


Figure 10: Effect of Clofibrate on Oral Glucose Tolerance

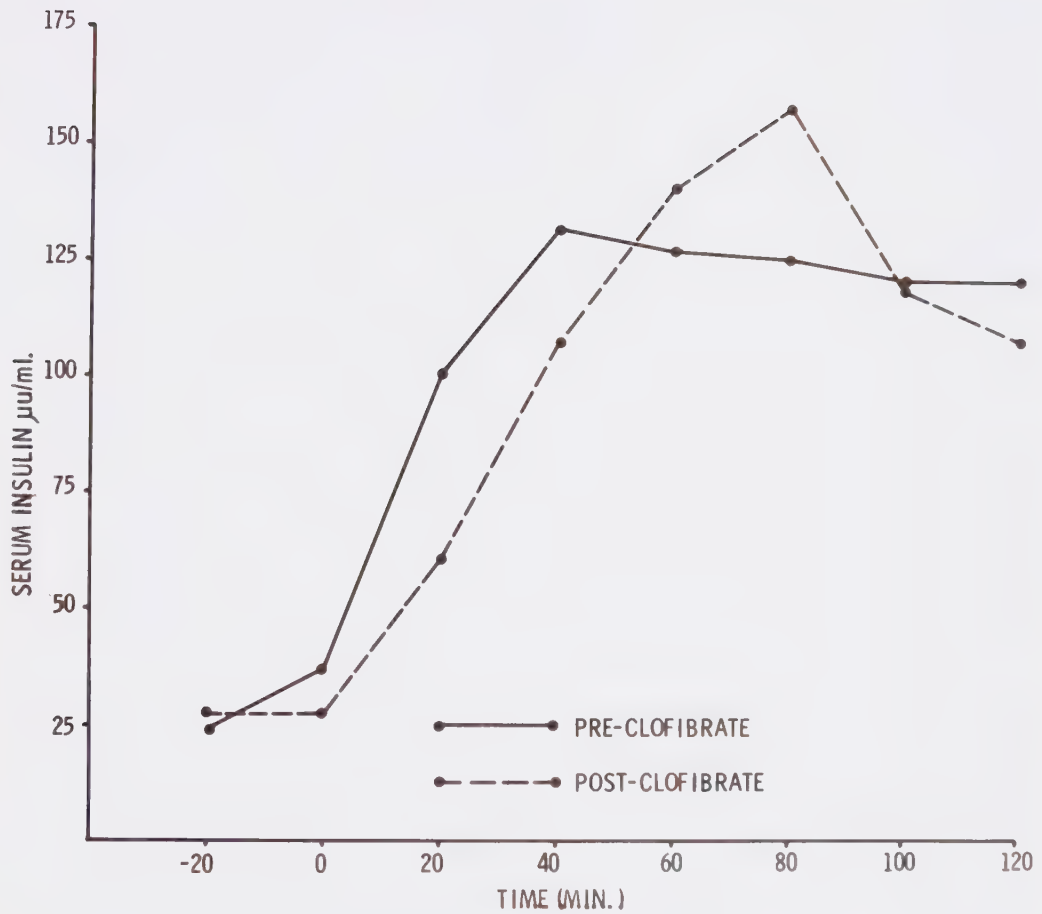


Figure 11: Effect of Clofibrate on Serum Insulin Levels
After Oral Glucose Administration

7. Plasma Glucose Studies After Fourteen Days of
Clofibrate Administration During Intravenous
Glucose Infusion

From the results noted above, it was felt that clofibrate might produce subtle changes in carbohydrate metabolism and serum insulin levels.

Because of the great variability in plasma glucose and serum insulin levels both before and after drug administration for thirty days, it was felt that intravenous glucose infusion might produce stable and reproducible insulin release by removing the variabilities of glucose absorption from the small intestine and by-pass the effect of the insulin-stimulating "gut factors"⁷³. In addition to the measurement of plasma glucose and serum insulin levels, it was decided to measure plasma free fatty acids during glucose infusion both before and after clofibrate administration.

Two subjects (P.K. and W.H.) were not available for this study and one was replaced by another volunteer (P.K.). The mean age of the group was 39 years with an age range of 24 - 49 years.

Table 21 and Figure 12 show the plasma glucose

values at 15 minute intervals on day zero and fourteen during the infusion of glucose intravenously at a rate of 500 ml/minute for 60 minutes. After fourteen days of clofibrate administration, plasma glucose values were lower at each 15 minute interval. These differences, however, are not statistically significant.

8. Serum Insulin Levels Before and After Clofibrate Administration for Fourteen Days

Table 21 and Figure 13 show the serum insulin levels obtained at day zero and fourteen during the infusion of glucose intravenously at a rate of 500 ml/min for 60 minutes. Serum insulin levels tended to be somewhat higher after fourteen days of clofibrate, but these differences are not statistically significant at any 15 minute interval. Serum insulin levels are also considerably lower than during oral glucose challenge, probably as a result of the lack of "gut factor" stimulation of insulin release.

9. Free Fatty Acid Levels Before and After Clofibrate Administration for Fourteen Days

Table 22 and 14 show the free fatty acid levels

TABLE 21

EFFECT OF FOURTEEN DAYS OF CLOFIBRATE ADMINISTRATION ON
PLASMA GLUCOSE AND SERUM INSULIN LEVELS DURING
INTRAVENOUS INFUSION OF GLUCOSE

Time (min)	Plasma Glucose mg/100 ml ± 2 S.D.		Serum Insulin μu/ml ± 2 S.D.	
	Day 0	Day 14	Day 0	Day 14
-15	96 ± 10	88 ± 10	11 ± 17	8 ± 22
0	94 ± 12	90 ± 8	8 ± 8	6 ± 11
15	126 ± 18	120 ± 15	17 ± 17	25 ± 24
30	146 ± 19	137 ± 28	33 ± 32	28 ± 34
45	162 ± 22	153 ± 34	31 ± 46	35 ± 48
60	173 ± 30	166 ± 32	45 ± 46	55 ± 90
75	143 ± 30	136 ± 40	51 ± 50	58 ± 90
90	122 ± 30	116 ± 36	33 ± 50	34 ± 68

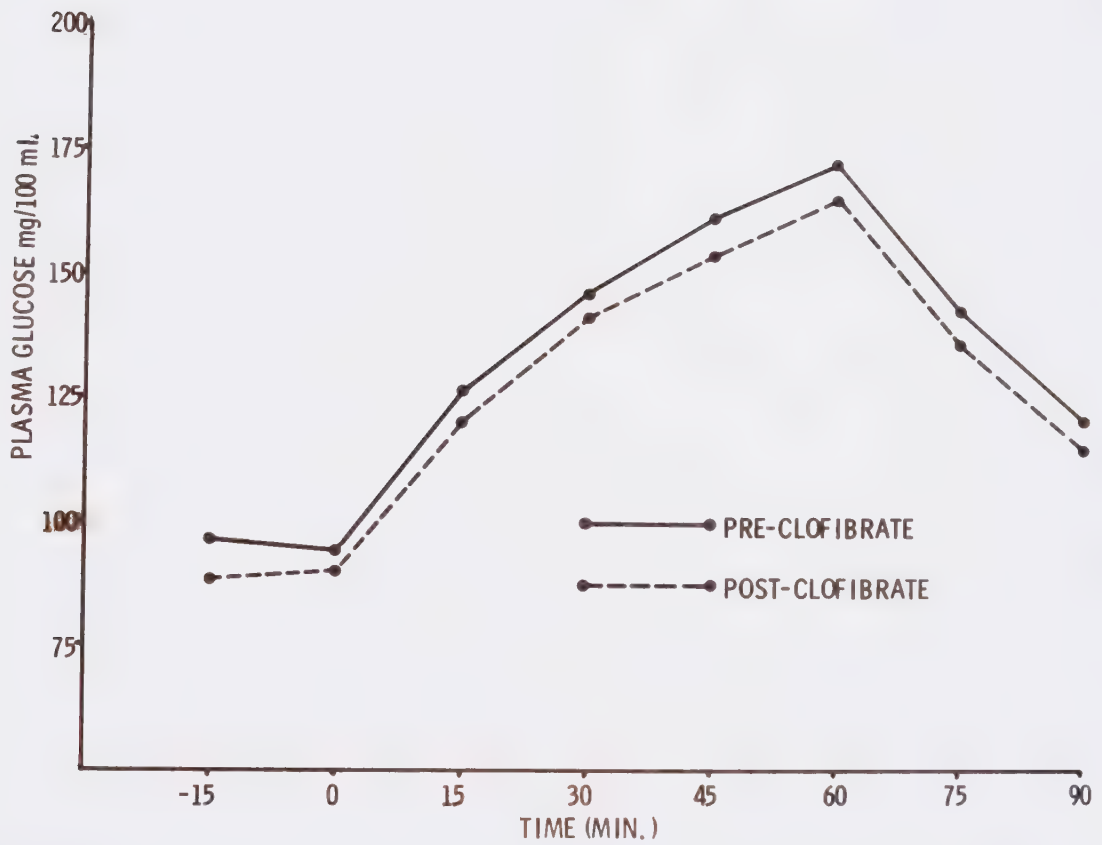


Figure 12: Effect of Clofibrate on Intravenous Glucose Administration

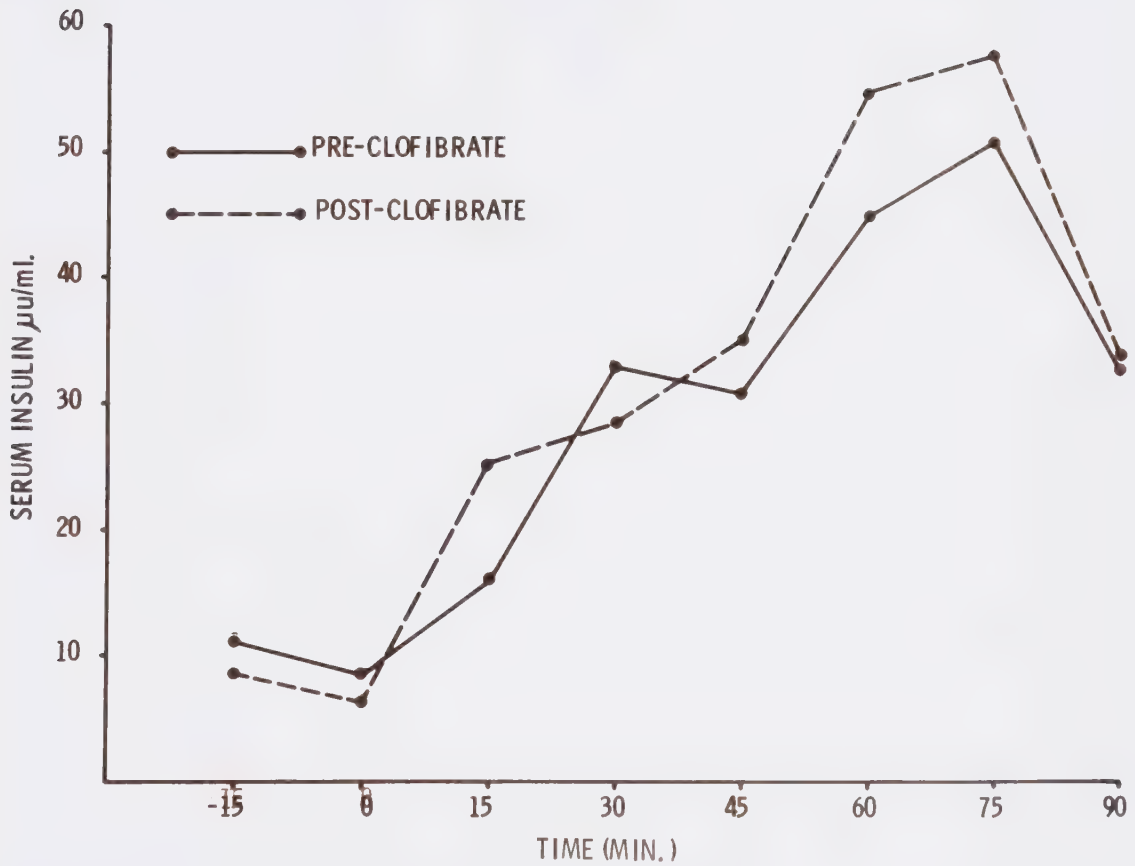


Figure 13: Effect of Clofibrate on Serum Insulin Levels
During Intravenous Glucose Infusion

TABLE 22

EFFECT OF FOURTEEN DAYS OF CLOFIBRATE ADMINISTRATION ON
FREE FATTY ACID LEVELS DURING INTRAVENOUS INFUSION
OF GLUCOSE

Time (min)	Free Fatty Acids mEquiv/L ± 2 S.D.	
	Day 0	Day 14
-15	636 ± 340	611 ± 222
0	595 ± 266	605 ± 226
15	598 ± 294	640 ± 260
30	528 ± 324	555 ± 174
45	462 ± 292	513 ± 142
60	414 ± 238	451 ± 128
75	351 ± 112	424 ± 108
90	344 ± 124	410 ± 76

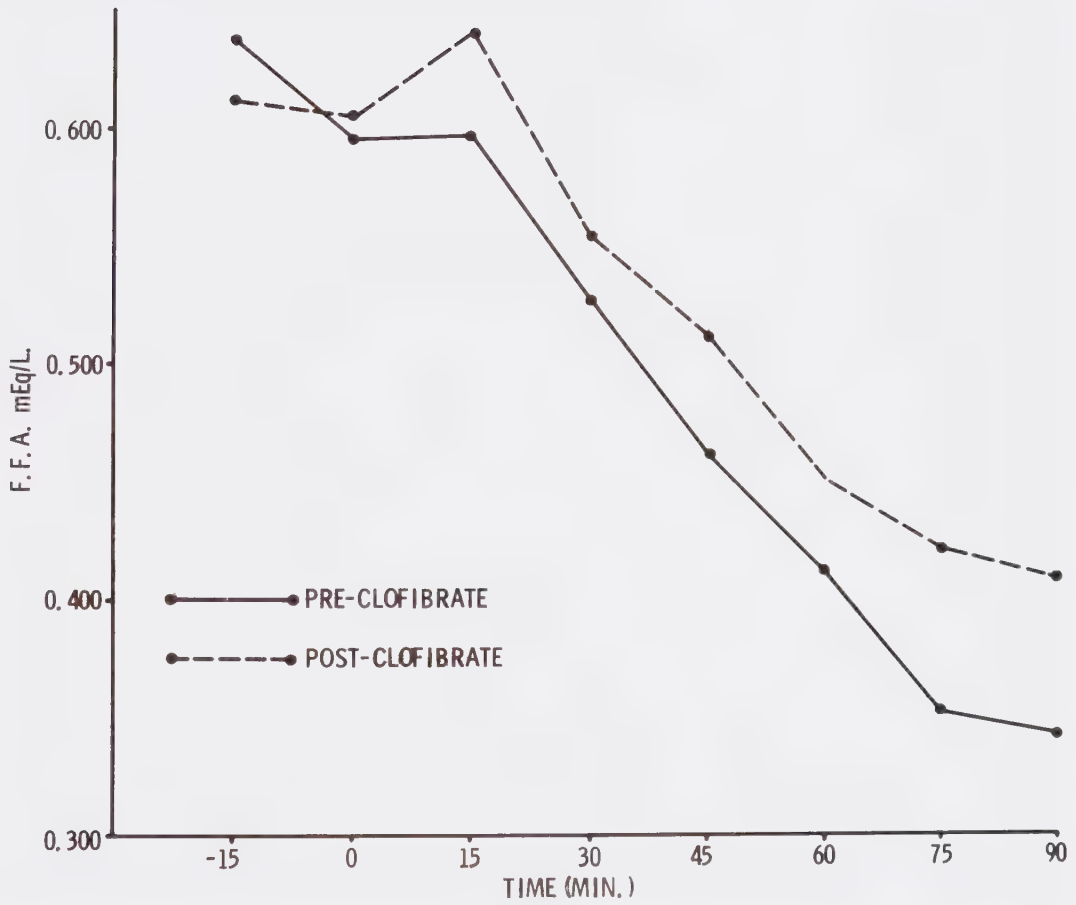


Figure 14: Effect of Clofibrate on Serum FFA's During Intravenous Glucose Infusion

obtained at day zero and fourteen during the infusion of glucose intravenously at a rate of 500 ml/minute for 60 minutes. Free fatty acid levels were higher at each 15 minute interval except -15 minutes, fourteen days after the initiation of drug therapy. These differences, however, are not statistically significant.

10. Serum Triglycerides, Cholesterol and Lipoproteins
After Fourteen Days of Clofibrate Administration

As essentially the same group of normal subjects was involved in the second study, it was decided to examine the effect of fourteen day clofibrate administration on serum triglycerides, cholesterol and lipoproteins. In Table 23 is shown a description of the subjects taking part in the fourteen day study. The data includes the age, serum triglyceride and cholesterol values and lipoprotein findings before and after clofibrate administration.

a) Serum Triglycerides

As seen in Table 24, the mean value for serum triglycerides prior to clofibrate administration was 224 mg/100 ml, and this fell to 146 mg/100 ml at the completion of fourteen days of

TABLE 23

THE EFFECT OF FOURTEEN DAYS OF CLOFIBRATE
ON THE SERUM LIPIDS OF NORMAL SUBJECTS

Pt.	Age (Yrs)	Triglycerides (mg/100 ml)		Cholesterol (mg/100 ml)	
		Day 0	Day 14	Day 0	Day 14
P.G.	48	255	180	155	153
G.V.	36	115	100	180	150
D.A.	44	95	75	177	158
A.B.	34	135	95	179	154
H.G.	48	245	105	148	145
A.M.	36	500	350	170	207
N.A.	49	390	180	185	162
P.K.	24	60	50	150	127

TABLE 24

AVERAGE EFFECT OF FOURTEEN DAYS OF CLOFIBRATE ON
THE SERUM TRIGLYCERIDES OF NORMAL SUBJECTS

Day	Range (mg/100 ml)	Mean (mg/100 ml)	Mean Decrease (mg/100 ml)
0	60 - 500	224	-
14	50 - 350	146	78

TABLE 25

AVERAGE EFFECT OF FOURTEEN DAYS OF CLOFIBRATE ON
THE SERUM CHOLESTEROL OF NORMAL SUBJECTS

Day	Range (mg/100 ml)	Mean (mg/100 ml)	Mean Decrease (mg/100 ml)
0	150 - 185	168	-
14	127 - 207	157	-11

therapy. This was a mean decrease of 78 mg/100 ml (35%), but was not statistically significant ($p < 0.3$) again due to the presence of A.M. in the group. However, this fourteen day study did reveal that the drug will reduce levels of serum triglycerides in individual patients in a short period of time.

b) Serum Cholesterol

The mean changes in serum cholesterol are given in Table 25. The mean value for cholesterol prior to clofibrate administration was 168 mg/100 ml. Numerically, this fell to 157 mg/100 ml after 14 days with a mean decrease of 11 mg/100 ml (7%) that is not statistically significant.

c) Serum Lipoproteins

By paper electrophoresis, 4/8 subjects had normal electrophoretic strips prior to drug administration (Table 26). Four of eight showed elevations of pre-beta lipoprotein, one man (A.M.) to a marked degree. At the end of 14 days, the 4/8 with normal strips before the drug showed no change. The 4/8 who demonstrated

TABLE 26

THE EFFECTS OF FOURTEEN DAYS OF CLOFIBRATE ON
THE SERUM LIPOPROTEINS OF NORMAL SUBJECTS

Pt.	Qualitative Paper Lipoprotein Evaluation		Qualitative Agarose Lipoprotein Evaluation	
	Day 0	Day 14	Day 0	Day 14
P.G.	slight elevation pre-beta	-	moderate elevation pre-beta	-
G.V.	-	-	-	reduced pre-beta
D.A.	-	-	-	reduced pre-beta
A.B.	-	-	-	reduced pre-beta
H.G.	slight elevation pre-beta	-	moderate elevation pre-beta	-
A.M.	marked elevation pre-beta	moderate elevation pre-beta	very marked elevation pre-beta	moderate elevation pre-beta
N.A.	moderate elevation pre-beta	slight elevation pre-beta	marked elevation pre-beta	-
P.K.	-	-	-	reduced pre-beta

elevated pre-beta lipoproteins showed qualitative reductions of the pre-beta fraction after clofibrate.

By agarose-gel electrophoresis, 4/8 showed normal electropherograms prior to drug administration (Table 26). Four of eight showed elevated pre-beta lipoprotein, one man (A.M.) to a marked degree. Following therapy, 7/8 showed normal or reduced amounts of pre-beta lipoprotein, while 1/8 (A.M.) showed qualitatively reduced amounts of pre-beta lipoprotein in comparison to his previous strip.

d) Changes in Lipoprotein Fractions as Revealed by Densitometric Scanning of Agarose-Gel Electropherograms

Table 27 shows the changes in relative percentages of the subjects' lipoprotein fractions following 14 days of clofibrate ingestion. The range, mean and mean change (increase or decrease) of the relative percentages of the lipoprotein fractions is shown in Tables 28, 29 and 30.

The mean value of pre-beta lipoprotein on day

TABLE 27

EFFECT OF FOURTEEN DAYS OF CLOFIBRATE ON SERUM LIPOPROTEIN
FRACTIONS AS SEPARATED BY AGAROSE GEL
ELECTROPHORESIS AND QUANTITATED BY DENSITOMETRIC SCANNING

Pt.	RELATIVE PERCENTAGE OF LIPOPROTEIN FRACTION					
	beta		pre-beta		alpha	
	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14
P.G.	41	43	41	37	18	20
G.V.	62	57	19	14	19	29
D.A.	71	67	11	7	18	26
A.B.	69	71	18	11	13	18
H.G.	47	60	36	15	17	25
A.M.	27	41	66	50	7	9
N.A.	36	57	54	26	10	17
P.K.	56	44	9	4	35	52

TABLE 28

CHANGES IN THE RATIO OF PRE-BETA TO OTHER LIPOPROTEIN

FRACTIONS AFTER FOURTEEN DAYS OF CLOFIBRATE

Day	Range of % Pre-beta Present	Mean of % Pre-beta Present	Decrease of Mean % of Pre-beta Present
0	9 - 66	32	-
14	4 - 50	21	-11

TABLE 29

CHANGES IN THE RATIO OF BETA TO OTHER LIPOPROTEIN

FRACTIONS AFTER FOURTEEN DAYS OF CLOFIBRATE

Day	Range of % Beta Present	Mean of % Beta Present	Increase of Mean % of Beta Present
0	27 - 71	51	-
14	41 - 71	55	4

TABLE 30

CHANGES IN THE RATIO OF ALPHA TO OTHER LIPOPROTEIN

FRACTIONS AFTER FOURTEEN DAYS OF CLOFIBRATE

Day	Range of % Alpha Present	Mean of % Alpha Present	Increase of Mean % of Alpha Present
0	7 - 35	17	-
14	9 - 52	25	8

zero (32%) was higher than at day fourteen (21%), but the difference is not significant ($p < 0.3$) (Table 28).

The mean value of beta lipoprotein on day zero (51%) was lower than at day fourteen (55%), but not significant ($p < 0.7$) (Table 29).

The mean value of alpha lipoprotein on day zero (17%) was lower than at day fourteen (25%), but not significant ($p < 0.2$) (Table 30).

DISCUSSION

A. THE EFFECT OF A SEVEN DAY HIGH CARBOHYDRATE DIET ON
SERUM LIPIDS IN NORMAL SUBJECTS AND PATIENTS WITH
CORONARY ARTERY DISEASE

At the present time, the spectrum of investigative procedures in patients with premature coronary artery disease includes relatively unsophisticated screening methods for lipid and carbohydrate abnormalities combined with angiographic and catheterization studies evaluating cardiac metabolism. Although useful, these investigations fall short in revealing subtle underlying metabolic defects which may contribute to the progression of the disease. Also, the recognition of such abnormalities has required prolonged periods of hospitalization with investigations carried out under precise steady-state conditions. Because of the overwhelming magnitude of this disease in the general population, such studies are not practical on a large scale. Thus, it was felt worthwhile to attempt to shorten and simplify these investigations so as to make them feasible for the general hospital setting. The approach used was as follows: lipoprotein measurements combined with a seven day period of high carbohydrate feeding were performed on patients with premature coronary artery

disease and a control group. Although the number of patients in this study is relatively small and subject to pre-selection on the basis of symptomatic coronary artery disease, the findings obtained from the study are illuminating and informative.

Initially, it was noted that the serum triglyceride levels of the patients with coronary artery disease were significantly higher than those of the control group. It was also noted, that elevation of the triglyceride fraction occurred more frequently than solitary elevation of serum cholesterol. These findings tend to support those of Albrink¹² and Carlson¹³, who found that triglyceride elevations were noted more consistently than cholesterol elevations in patients with coronary artery disease under the age of 50. However, more significantly, the patient group showed a much greater increase in serum triglyceride values than did the controls after high carbohydrate feeding. Also, by adopting the proposition that marked elevation of the pre-beta lipoprotein after 7 days of high carbohydrate feeding reflects abnormal carbohydrate-inducibility, 14 of the 21 patients (66%) demonstrated the phenomenon. Endogenous hypertriglyceridemia has been shown to be a variable characteristic that can be produced in most individuals by high carbohydrate feeding. The mechan-

isms responsible for this occurrence are incompletely understood and the discrimination of normal and abnormal responses remains an area of uncertainty. Lees and³² Fredrickson³², have suggested that an increase in serum triglycerides of 400 mg/100 ml in seven to ten days on a diet containing 7 gm/kg/day of carbohydrate should be considered an abnormal response. In the work presented here, there is good evidence to suggest that an increase in serum triglycerides of 100 - 150 mg/100 ml in seven days reflects an abnormal response.

Although serum cholesterol levels prior to carbohydrate challenge were significantly higher in the patient group; mean levels were within the accepted limits of normal for the age range of the patient population. Following the diet; serum cholesterol fell in both groups, but to a greater degree in the patient group. This may be explained by the higher mean cholesterol level in the patient group before the diet was initiated.

The changes noted in serum triglycerides during the dietary period correlated well with the changes observed by lipoprotein electrophoresis. It was noted that although about one-half of the patient group of 21 had normal electropherograms prior to the dietary study, only five

remained normal at the completion of the dietary challenge. Thus, the diet appeared to uncover latent carbohydrate inducibility in about one-half of the patients that were normal prior to the initiation of the diet. It was also noted, that the degree of elevation of triglycerides appeared to correlate well with the changes noted qualitatively on lipoprotein electrophoresis, particularly when the lipoproteins were separated on agarose.

Densitometric scanning of the lipoprotein fractions by agarose-gel electrophoresis revealed much lower relative amounts of pre-beta lipoprotein in the control group than in the patient group prior to the period of dietary challenge. This is a logical finding when it is recalled that the control group had much lower baseline triglyceride values. Following 7 days of high carbohydrate feeding, the relative amount of pre-beta lipoprotein in both normal subjects and patients increased significantly. The patient group had a larger increase numerically, but this was not statistically significant. There were no significant changes in the relative amounts of beta lipoprotein in either group following a high carbohydrate diet. The relative percentage differences of alpha

lipoprotein between the control and patient group is not readily explainable but may suggest that the higher levels noted in the control group is due to a relatively lower amount of pre-beta lipoprotein present, both before and after the dietary period.

Abnormalities of glucose tolerance in the patient population are somewhat less frequent than have been reported in other studies of this nature. This is perhaps due to the relatively more rigid criteria established for glucose intolerance in this study. In the patient group, only 19% demonstrated abnormalities of glucose tolerance as expressed by the glucose tolerance index. This method of expression is felt to be the best single expression of the peak and duration of the glucose tolerance curve.

Although not physiological by present dietary standards, this seven day period of high carbohydrate feeding is analogous to other forms of stress employed to elicit latent metabolic abnormalities and may be compared to the cortisone glucose tolerance test. It is felt that this method of study must form part of the routine investigation of young patients with coronary artery disease. It has become abundantly clear that many

patients, some included in this study, have normal or only slightly elevated levels of serum triglycerides, but when exposed to high carbohydrate feeding will respond to an abnormal degree. The importance of elucidating this phenomenon lies in the long term management of such patients. Formerly, with the empirical reduction of cholesterol intake following myocardial infarction or acute coronary insufficiency, the amount of carbohydrate in the diet is necessarily increased to maintain adequate caloric intake. Increasing the amount of dietary carbohydrate in an individual susceptible to carbohydrate induction will only serve to perpetuate and perhaps hasten the development of progressive vascular disease. It becomes clear that individuals with this propensity should be managed with diets low in carbohydrates. It is interesting to speculate on the significance of the triglyceride response in two of the subjects in the control group (H.S. and R.B.) who showed a marked triglyceride increase when placed on the diet. Both subjects had normal triglyceride levels initially. Because of the prevalence of carbohydrate-inducibility in young patients with coronary artery disease, it may be that this characteristic is an indicator at an early

age of a tendency to develop ischemic heart disease prematurely. Thus, it would be of interest to follow such individuals in future years for the development of coronary artery disease.

The diet has proven to be effective, palatable, and easy to prepare and administer to patients. Because of its liquid nature, it lends itself well for use on an outpatient basis.

B. THE EFFECTS OF CLOFIBRATE ON NORMAL SUBJECTS

There are many reports of the effect of clofibrate on patients with hyperlipidemia. However, there has not been a study done of the effect on serum lipids and lipoproteins in apparently healthy subjects.

This work has shown that clofibrate administered for 30 days will numerically reduce serum triglycerides and cholesterol in normal males. However, the degree of reduction does not appear to be statistically significant. This is in part explained by the small numbers in the group studied, as well as the inclusion of the data from A.M., who in retrospect had previously unrecognized and severe hyperpre-beta lipoproteinemia with a serum triglyceride level of 770 mg/100 ml prior to the administration of clofibrate. Exclusion of this man from the groups results in a reduction by clofibrate of the mean triglycerides for the group that is statis-

tically significant ($p < 0.05$). Serum cholesterol levels, while reduced, did not change significantly. This would be expected for two reasons; the fact that clofibrate has a less consistent effect on beta lipoproteins and that serum cholesterol was near the lower limits of normal before the drug was administered. This study also demonstrated the superiority of agarose gel electrophoresis in evaluating subtle changes in the pre-beta fraction of subjects before and after drug administration. The relative reduction or absence of pre-beta lipoproteins on agarose-gel following clofibrate could not be appreciated by paper electrophoresis. This would indicate that in studies involving the evaluation of subtle changes in pre-beta lipoproteins, the agarose-gel method is an important technical advance and the method of choice for studying the pre-beta fraction by electrophoresis.

The relative percentage changes of the pre-beta, beta and alpha fractions as quantitated by densitometric scanning are substantial numerically, particularly for the pre-beta fraction but not statistically significant. The principle effect of clofibrate is to reduce levels of VLDL. Because of the relationship of this fraction

to carbohydrate metabolism and insulin, it was decided to compare serum insulin levels following glucose administration, before and during administration of the drug.

Plasma glucose levels following the administration of 100 gm of glucose orally in the fasting state after clofibrate administration, were higher than those obtained prior to drug therapy. These findings were consistent at each twenty minute interval for two hours but were not statistically significant. This is, in part, due to the wide variation of individual values between the subjects tested leading to a rather large standard deviation at each twenty minute interval. Serum insulin levels of the patients on clofibrate, following the administration of glucose orally were lower at 20 and 40 minutes and higher at 60 and 80 minutes when compared to the values obtained prior to drug administration.

From the results above, it was considered that the administration of clofibrate might produce subtle changes in carbohydrate metabolism. It was felt that the study should be repeated, but using intravenous glucose challenge to remove the variation in the absorption of glucose orally and overcome the effects

73

of "gut factors" which affect serum insulin release. Once again, the drug, when administered for fourteen days produced a numerical reduction in serum triglycerides and cholesterol levels. The results of densitometric scanning of the lipoprotein fractions by agarose-gel electrophoresis were similar to the previous oral glucose study. Plasma glucose levels during the infusion tended to be lower after administration of the drug than before, but the differences are not statistically significant. Plasma FFA values, during the intravenous glucose infusion were numerically higher after clofibrate administration tending to contradict statements that the drug lowers FFA in the plasma. This study has thus shown that clofibrate will lower serum triglyceride and serum cholesterol levels (the latter to a lesser degree) in normal male subjects. It has also been useful in emphasizing the advantages of agarose-gel electrophoresis in evaluating changes in pre-beta lipoproteins. Finally, it must be stated that within the limits of the study, clofibrate has no statistically demonstrable effects on plasma glucose and serum insulin levels following oral and intravenous glucose challenge.

SUMMARY

A. DIETARY STUDY

The purpose of this study was to assess a group of young males with well-documented coronary artery disease for abnormalities in serum lipoproteins (particularly the VLDL), carbohydrate intolerance and inducibility of endogenous hypertriglyceridemia by carbohydrate feeding. A seven day period of high carbohydrate diet was assessed for its adequacy to detect this latter abnormality.

Accordingly, twenty-one males under 50 years of age with coronary disease were compared with 9 control subjects under 30 years of age with regard to serum lipid levels, serum lipoproteins (studied by paper and agarose-gel electrophoresis) and lipid response to a seven day period of high carbohydrate feeding. The results of this study may be summarized as follows:

- 1) As a group, the serum triglycerides were significantly higher in the patients than in the controls even before the carbohydrate challenge. The increase of serum triglycerides in the patient group was also much greater than in the control group.

- 2) Solitary elevation of serum cholesterol was an uncommon finding in the patient group suggesting that in the age group under consideration, triglycerides are a better indicator of risk for the development of coronary artery disease.
- 3) A large proportion of the patients (66%), demonstrated carbohydrate inducibility following high carbohydrate feeding confirming the frequency of this phenomenon in young patients with coronary artery disease.
- 4) A period of seven days of high carbohydrate feeding is adequate to demonstrate latent carbohydrate inducibility and is practical for use in a large active treatment hospital and should form part of the investigation of young patients with coronary artery disease.
- 5) The diet, when prepared in liquid form can be successfully administered on an outpatient basis, thus overcoming the need for complicated dietary preparation.
- 6) The phenomenon of carbohydrate inducibility may be present long before overt coronary artery

disease is manifest.

B. CLOFIBRATE STUDY

A second aspect of this research was to evaluate the effect of clofibrate on the serum lipids and lipoproteins of normal males. In addition to evaluating lipid responses, the possible effect of the drug on serum insulin, glucose tolerance and serum FFA was studied following oral and intravenous glucose administration before and after clofibrate therapy. The results of this study may be summarized as follows:

- 1) The drug will lower both serum triglyceride and cholesterol levels in normal males. The greatest reductions appear to be in those subjects with the higher baseline values.
- 2) Clofibrate decreases the relative amount of pre-beta lipoprotein present in normal individuals.
- 3) No significant effect of the drug can be noted on glucose tolerance, serum insulin and serum free fatty acid levels following 30 days of clofibrate administration.
- 4) Agarose-gel electrophoresis is the method of choice for studying subtle changes in the VLDL

by electrophoretic techniques.

APPENDIX

STATISTICAL ANALYSIS

A. Dietary Studies

The differences in serum lipids between the control and patient groups due to diet were analyzed utilizing the unpaired t-test.⁷⁴

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{(x_{1i} - \bar{x}_1)^2 + (x_{2i} - \bar{x}_2)^2}{N_1 + N_2 - 2} \left(\frac{1}{N_1} + \frac{1}{N_2} \right)}}$$

N_1 = size of sample 1

N_2 = size of sample 2

Degrees of freedom = $N_1 + N_2 - 2$

B. Clofibrate Study

The effects of clofibrate on any given parameter (serum lipids, lipoprotein fractions, serum insulin and serum F.F.A.'s) were analyzed utilizing the paired t-test.⁷⁴

$$t = \frac{d^2}{\sqrt{\frac{(d_i - \bar{d})^2}{N(N-1)}}}$$

Degrees of freedom = $N - 1$

BIBLIOGRAPHY

1. Marchand, F.: Ueber Athero-Sklerose, Verhandl. d. Kongr. f. inn. Med. 21: 23, 1904.
2. Anitschkow, N., Chatalow, S.: Ueber Experimentelle Cholesterinsteatose und ihre Bedeutung fur die Entstehung einiger. Pathologischer Prozesse. Centralbl. Allg. Path. Path. Anat. 24: 1, 1913.
3. Kannel, W.B., Dawber, T.R., Kagan, A., Revotskie, N., Stokes, J. III.: Factors of risk in the development of coronary artery disease - six year follow-up experience - The Framingham Study. Ann. Int. Med., 55: 33, 1961.
4. Chapman, J.M., Massey, F.J.: The interrelationship of serum cholesterol, hypertension, body weight, and coronary artery disease. Results of the first ten years of the Los Angeles Heart Study. J. Chronic. Dis. 17: 933, 1964.
5. Keys, A., Taylor, H.L., Blackburn, H., Brozek, J., Anderson, J.T., Simonson, E.: Coronary heart disease among Minnesota business and professional men followed fifteen years. Circulation 28: 381, 1963.
6. Paul, O., Kepper, M.H., Phelan, W.H., Dupertius, G.W., MacMillan, A., McKean, H., Park, H.: A longitudinal study of coronary artery disease. Circulation 28: 20, 1963.
7. Epstein, F.: Epidemiology of coronary artery disease. J. Chronic Dis. 18: 735, 1965.
8. Gofman, J., Strisower, B., De Lalla, O., Tamplin, A., Jones, H., Lindgren, F.: Index of coronary artery atherogenesis. Mod. Med. 11: 119, 1953.
9. Brown, D.F., Kinch, S.H., Doyle, J.T.: Serum triglycerides in health and ischemic heart disease. N.E.J.M. 273: 947, 1965.
10. Doyle, J.T.: Risk factors in coronary artery disease. Mod. Concepts of C.V. Disease 35: 81, 1966.
11. Albrink, M.J., Man, E.B.: Serum triglycerides in coronary heart disease. Arch. Int. Med. 103: 4, 1959.

12. Albrink, M.J.: Triglycerides, lipoproteins and coronary artery disease. Arch. Int. Med. 109: 145, 1962.
13. Carlson, L.: Serum lipids in men with myocardial infarction. Acta. Med. Scand. 167: 399, 1960.
14. Havel, R.J., Carlson, L.A.: Serum lipoproteins, cholesterol and triglycerides in coronary artery disease. Metabolism 11: 195, 1962.
15. Albrink, M.J.: Carbohydrate metabolism and cardiovascular disease. Ann. Int. Med. 62: 1330, 1965.
16. Reaven, G.A., Calciano, R., Cody, C., Lucas, C., Miller, R.: Carbohydrate intolerance and hyperlipemia in patients with myocardial infarction without known diabetes mellitus. J. Cl. Endocrinology 23: 1013, 1963.
17. Davidson, P.C., Albrink, M.J.: Insulin resistance in hyperglyceridemia. Metabolism 14: 1059, 1965.
18. Ostrander, L.D., Jr., Francis, T., Hayner, N.S., Kjelsburg, M.O., Epstein, F.H.: The relationship of cardiovascular disease to hyperglycemia. Ann. Int. Med. 62: 1188, 1965.
19. Kane, J.P., Loncope, C., Pavlatos, F.C., Grodsky, G.M.: Studies of carbohydrate metabolism in idiopathic hypertriglyceridemia. Metabolism 14: 471, 1965.
20. Knittle, J.L., Ahrens, E.H., Jr.: Carbohydrate metabolism in two forms of hyperglyceridemia. J. Clin. Invest. 43: 485, 1964.
21. Farquhar, J.W., Frank, A., Gross, R.C., Reaven, G.M.: Glucose, insulin and triglyceride responses to high and low carbohydrate diets in man. J. Clin. Invest. 45: 1648, 1966.
22. Reaven, G.M., Lerner, R.L., Stern, M.P., Farquhar, J.W.: Role of insulin in endogenous hypertriglyceridemia. J. Clin. Invest. 46: 1756, 1967.
23. Salans, L.B., Reaven, G.W.: Effect of insulin pretreatment on glucose and lipid metabolism of liver slices from normal rats. Proc. Soc. Exptl. Biol. Med. 122: 1208, 1966.

24. Falsetti, H.L., Schnatz, J.D., Greene, D.G., Bunnell, I.L.: Lipid and carbohydrate studies in coronary artery disease. *Circulation* 37: 184, 1968.
25. Tzagournis, M., Chiles, R., Ryan, J.M., Skillman, T.G.: Interrelationships of hyperinsulinism and hypertriglyceridemia in young patients with coronary heart disease. *Circulation* 38: 1156, 1968.
26. Mahler, R.: Diabetes and arterial lipids. (Abstract) *Quart. J. Med.* 34: 484, 1965.
27. Stout, R.W.: Insulin-stimulated lipogenesis in arterial tissue in relation to diabetes and atheroma. *Lancet* 2: 702, 1968.
28. Hatch, F.T., Abell, L.L., Kendall, F.E.: Effects of restriction of dietary fat and cholesterol upon serum lipids and lipoproteins in patients with hypertension. *Am. J. Med.* 19: 48, 1955.
29. Ahrens, E.H., Jr., Hirsch, J., Oette, K., Farquhar, J., Stein, Y.: Carbohydrate and fat-induced lipemia. *Trans. Ass. Amer. Physicians* 74: 134, 1961.
30. Kuo, P.T.: Dietary sugar in the production of hypertriglyceridemia. *Ann. Int. Med.* 62: 1199, 1965.
31. MacDonald, I., Braithwaite, D.M.: Influence of dietary carbohydrate on lipid patterns in sera and in adipose tissue. *Clin. Sci.* 27: 23, 1964.
32. Lees, R.S., Fredrickson, D.S.: Carbohydrate induction of hyperlipemia in normal man. *Clin. Research* 13: 327, 1965.
33. Blankenhorn, D.H., Chin, H.P., Lau, F.Y.K.: Ischemic heart disease in young adults. *Ann. Int. Med.* 69: 21, 1968.
34. Nerking, J.: *Pflueger Arch. ges. Physiol.* 85: 330, 1901.
35. Macheboeuf, M.M.A.: *Bull. soc. chim. biol.* 11:268, 1929.

36. Gofman, J.W., Lindgren, F.T., Elliott, H.: Ultra-centrifugal study of lipoproteins of human serum. *J. Biol. Chem.* 179: 973, 1949.
37. Lewis, L.A., Green, A.A., Page, I.H.: Ultracentrifuge lipoprotein patterns of serum of normal, hypertensive and hypothyroid animals. *Am. J. Physiol.* 171: 391, 1952.
38. Hazelwood, R.N.: Molecular weights and dimensions of some high-density human serum lipoproteins. *J. Am. Chem. Soc.* 80: 2152, 1958.
39. Bjorklund, R., Katz, S.: Molecular dimensions and weights of some human serum lipoproteins. *J. Am. Chem. Soc.* 78: 2122, 1956.
40. Fredrickson, D.S., Levy, R.I., Lees, R.S.: Fat transport in lipoproteins - an integrated approach to mechanisms and disorders. *N.E.J.M.* 276: 32-44, 94-103, 148-156, 215-226, 273-281, 1967.
41. Lees, R.S., Hatch, F.T.: Sharper separation of lipoprotein species by paper electrophoresis in albumen-containing buffer. *J. Lab. and Clin. Med.* 61: 518 - 528, 1963.
42. Henry, R., Berkman, S., Golub, O., Segalove, M.: The hyperlipoproteinemias by paper electrophoresis. *Handbook of special diagnostic laboratory tests.* Bio-Science Laboratories, Van Nuys, California: 115, 1969.
43. Bierman, E.L., Gordis, E., Hamlin, J.T. III.: Heterogeneity of fat particles in plasma during alimentary lipemia. *J. Clin. Invest.* 41: 2254, 1962.
44. Durrum, E.L., Paul, M.H., Smith, E.R.B.: Lipid detection in paper electrophoresis.: *Science* 116: 428, 1962.
45. Smith, E.B.: Lipoprotein patterns in myocardial infarction: relationship between components identified by paper electrophoresis and in the ultra centrifuge. *Lancet* 223: 910, 1957.

46. Chin, H.P., Blankenhorn, D.H.: Separation and quantitative analysis of serum lipoproteins by means of electrophoresis on cellulose acetate. Clin. Chem. Acta. 20: 305, 1968.
47. Irwin, W.C., Campbell, D.J.: Ultracentrifugation and electron microscopy characterization of human serum lipoproteins as separated by agarose gel electrophoresis. (Abstract) Clin. Chem. 14: 47, 1968.
48. Ewing, A.M., Freeman, N.K., Lindgren, F.T.: The analysis of human serum lipoprotein distributions. Advances in Lipid Research 3: 25, 1965.
49. Fredrickson, D.S., Levy, R.I., Lindgren, F.T.: A comparison of heritable abnormal lipoprotein patterns as defined by two different techniques. J. Clin. Invest. 47: 2446, 1968.
50. Mancini, G., Carbonara, A.O., Heremans, J.F.: Method of single radial immunodiffusion. Immunochemistry 2: 235, 1965.
51. Oliver, M.F.: Effects of Atomid and CPIB on serum lipids. J. Atheroscler. Res. 3: 427, 1963.
52. Thorp, J.M., Waring, W.S.: Modification of metabolism and distribution of lipids by ethyl chlorophenoxyisobutyrate. Nature 194: 948, 1962.
53. Oliver, M.F.: Reduction of serum lipid and uric acid levels by an orally active androsterone. Lancet 1: 1321, 1962.
54. Hellman, L., Zumoff, B., Kessler, G., Rosenfeld, R., Gallagher, T.F.: Reduction of serum cholesterol in man by an oral androsterone preparation. J. Clin. Invest. 41: 364, 1962.
55. Strisower, E.H.: The response of hyperlipoproteinemias to atomid and ethyl chlorophenoxyisobutyrate. J. Atheroscler. Res. 3: 445, 1963.
56. Oliver, M.F., Roberts, S.D., Hayes, D., Pentridge, J.F., Suzman, M.M., Bersohn, I.: Effect of atomid and ethyl chlorophenoxyisobutyrate on anticoagulant requirements. Lancet 1: 143, 1963.

57. Cotton, R.C., Wade, E.G., Spiller, G.W.: The effect of atomid on plasma fibrinogen and heparin resistance. *J. Atheroscler. Res.* 3: 648, 1963.
58. Carson, P., McDonald, L., Pickard, S., Pilkington, T., Davies, B., Love, F.: Effect of atomid on platelet stickiness. *J. Atheroscler. Res.* 3: 619, 1963.
59. Glynn, M.F., Murphy, E.A., Mustard, J.F.: Effect of clofibrate on platelet economy in man. *Lancet* 1: 449, 1967.
60. Hellman, L., Zumoff, A., Kessler, G., Kara, E., Rubin, I. L., Rosenfeld, R.S.: Reduction of cholesterol and lipids in man by ethyl p-chlorophenoxyisobutyrate. *Ann. Int. Med.* 59: 477, 1963.
61. Thorp, J.M.: An experimental approach to the problem of disordered lipid metabolism. *J. Atheroscler. Res.* 3: 351, 1963.
62. Duncan, D.H., Best, M.M., Despopoulos, A.: Inhibition of hepatic secretion of triglyceride by chlorophenoxyisobutyrate (CPIB). *Circulation Supplement* 3: 7, 1964.
63. Spritz, N.: Effects of ethyl - α -p-chlorophenoxyisobutyrate (CPIB) on endogenous hyperglyceridemia. *Circulation Suppl.* 2: 201, 1965.
64. Azarnoff, D.L., Tucker, D.R., Barr, G.A.: Studies with ethyl chlorophenoxyisobutyrate (clofibrate). *Metabolism* 14: 959, 1965.
65. Langer, R., Levy, R.I.: Acute muscular syndrome in patients receiving clofibrate. *N.E.J.M.* 279: 856, 1969.
66. Zakim, D., Herman, R.H., Anderson, J.W.: Effect of Atomid-S on the relation between plasma insulin and triglyceride concentration in carbohydrate-induced hypertriglyceridemia. *Cl. Res.* 15: 333, 1967.
67. Black, W.D., Jarrett, J.K., Jr., Levine, J.B.: Automation in analytical chemistry, *Technicon Symposia*, Mediad Inc. N.Y.: 345 - 347, 1965.

68. Kessler, G., Lederer, H.: Fluorometric measurement of triglycerides. Automation in analytical chemistry. Technicon Symposia, Mediad Inc. N.Y.: 341 - 345, 1965.
69. Skeggs, L.T.: Simultaneous glucose/BUN. Technicon Symposia, Mediad Inc. N.Y.: 166, 1965.
70. Morgan, C.R., Lazarow, A.: Immunoassay of insulin: two antibody system. Diabetes 12: 115, 1963.
71. Trout, D.L., Estes, E.H., Friedburg, S.J.: Titration of free-fatty acids of plasma. J. Lipid Research 1: 199, 1960.
72. Ostrander, L.D., Neff, B.J., Block, W.D., Francis, T., Epstein, F.H.: Hyperglycemia and hypertriglyceridemia among persons with coronary artery disease. Ann. Int. Med. 67: 34, 1969.
73. Dupre, J., Curtis, J.D., Waddell, R.W., Beck, J.C.: Regulation of pancreatic endocrine function by gastrointestinal hormones. Proc. Roy. Soc. Med. 61: 815, 1968.
74. Bradford Hill, A.: Principles of medical statistics. Oxford University Press, New York, 1967.

B29921